The Journal of Parasitology

Volume XVI SEPTEMBER, 1929 Number 1

STUDIES ON AMOEBAE FROM HUMAN HOSTS

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In 1921 Kofoid and Swezey announced a new species of intestinal amoeba from man to which they gave the name *Councilmania lafleuri*. To this new form were attributed certain peculiarities said to be found in no other intestinal amoeba. When the description given is compared to that commonly accepted for *Endamoeba coli* it can be seen that the two are very similar, and this resemblance is, indeed, the cause of the controversy as to whether *C. lafleuri* is a new species. According to the California authors the genus Councilmania differs from all other intestinal amoebae in the occurrence in fresh stools of a reproductive process of repeated gemmation resulting in the escape of amoebulae from the cyst. The process has been designated "budding" and is said to be preceded by the formation of a ridge or intra-cystic process which results in a protrusion of protoplasm through a small opening in the cyst wall, in the escape of a nucleus into this lobe, and in the subsequent detachment of an amoebula, to be followed by a repetition of the process until the cyst is emptied of all its nuclei.

It is the purpose of the present paper to describe and to interpret some observations and experimental work carried out on human intestinal amoebae and their eight-nucleated cysts in the light of the claims of Kofoid and Swezy as to the character of *Councilmania lafleuri* and the opinions of those who contradict those claims.

This investigation was carried out under the supervision of Dr. David H. Wenrich in the Zoological Department of the University of Pennsylvania. The author wishes to thank Dr. Wenrich for his guidance and many thoughtful suggestions and criticisms, which were a great aid in the pursuit of this work.

MATERIAL AND METHODS

The material used in these experiments came for the most part from human hosts whose stools showed either the presence of amoebae or cysts. Fresh material has been difficult to obtain, most specimens being from one to several days old upon arrival. In one case, however, it was possible to make a series of fixed smears for staining within five minutes after the stool was passed and from this very interesting results were obtained. The rats used in the experiments were born in the vivarium of this laboratory, their parents being Wistar Institute rats.

Professor Kofoid was kind enough to send to Dr. Wenrich several slides that he had diagnosed as *Councilmania lafleuri* and these were made available to the writer for study. Thanks are due to Dr. A. D. Waltz, Pathologist of the Children's Hospital, Philadelphia, for his co-operation in securing some of the material used in this study.

The examination of all fresh material was made in 0.6% salt solution. Stained slides were made of all in which findings were positive. The fixatives used were chiefly warm Schaudinn's fluid and picro-mercuric, the latter made up according to Yocum's formula. Iron hematoxylin was used for staining. It was customary to isolate the rats without food or water a day before feeding. Washed cysts were then suspended in milk and fed to the rats.

OBSERVATIONS AND RESULTS

The first observations were made on fixed and stained slides of human feces in which the vegetative forms of an amoeba were found (Case No. 52).

In these amoebae there was no marked distinction between endoplasm and ectoplasm. The endoplasm was slightly vacuolated. On the inner surface of the nuclear membrane particles of chromatin were arranged in a relatively thin layer giving in some instances a crenate inner margin to the periphery of the nucleus. The karyosome was more or less eccentric in position, spherical in shape and, in some instances, with a clear halo surrounding it. The pericaryosomal area was generally clear, although in some nuclei traces of a linin network and a few scattered chromatin granules were noticed. The amoebae ranged from 21 to 25μ, averaging 23μ in diameter. In a thorough examination of 15 slides no cysts were found nor were the vegetative forms very numerous.

Later from this case it was possible to get a fresh specimen immediately after passage for a second series of observations. Smears of the stool were made and fixed before five minutes had elapsed, and an examination of the unstained and unfixed material was also made. In the fresh material the amoebae present were large and striking in the fact that clear, hyaline pseudopodia were thrust out suddenly and the superficial resemblance to *E. histolytica* was marked. However, a more thorough examination was made of the fixed and stained slides. No appreciable difference was noticed in the material due to difference in fixatives. The amoebae, which were much more numerous in this series than in the other, exhibited the same nuclear characteristics as did those of the first series described above, except that some had no distinct caryosomes. In addition most of them possessed clear, broad, rounded pseudopodia with marked distinction between ectoplasm and

endoplasm (Fig. 3.). The average size of these amoebae was 25μ, ranging from 21 to 27μ in diameter. Quite a few showed two nuclei.

No eight-nucleated cysts were found but a number of two and four-nucleated cysts resembling those of *E. coli* were present (Figs. 1 and 2). The two-nucleated cysts were much more numerous than the four-nucleated in the ratio of about 8:1, both types ranging in diameter from 12 to 16μ. The cysts had a distinct wall and were mostly spherical in shape, but some varied, being oval or ellipsoidal. The nuclear membranes of the cyst forms were thin, and in some cases the space between the nuclear membrane and the karyosome stained very lightly. The karyosome was generally central in position and spherical in shape. In their small size and prominent glycogen vacuole (Fig. 1) the two-nucleated cysts resembled figures given by Kofoid and Swezy for *C. lafleuri.* Some of the slides of this series were not fixed immediately but were made from the material after it had been standing for varying periods of time up to one hour or more. An examination of the vegetative forms in these slides show little or no difference between ectoplasm and endoplasm and the amoebae were much reduced in number (Fig. 4). Here, then, may be an explanation for the scarcity and appearance of the vegetative forms in the earlier series since that series was made from material more than one hour old. There was in this case a definite association between amoebae with clear ectoplasmic pseudopodia but with nuclei like those described for *C. lafleuri,* and binucleate and quadrinucleate cysts which were of the *E. coli* type. In this series of slides from case 52 there were a good many individuals of *Dientamoeba fragilis,* but there was no confusion between them and the larger amoebae just described.

Shortly after the above observations were recorded another stool was sent to the laboratory (Case no. 80) containing eight-nucleated coli-like cysts. Smears were made from this case and fixed in both Schaudinn's fluid and picromercuric. Upon examination much interest was aroused because the cysts fixed in warm Schaudinn's fluid showed little evidence of "budding" while the cysts fixed in picromercuric showed "budding" such as Kofoid and Swezy (1921) attribute to Councilmania. In a count of 100 cysts on a slide fixed in Schaudinn's fluid four showed either a "bud" or a chromophile ridge to ninety-six that showed neither. On another slide fixed in picromercuric a count of 100 cysts included ninety with a "bud" or ridge to ten which did not.

It was decided to investigate this matter further in an endeavor to produce "budding" by other means. A supply of washed cysts was prepared and the experiments to induce "budding" carried out on these First the effect of pressure upon the cyst wall was tried (Gunn 1922). Some of the cysts were placed in a small amount of salt solution on a slide and covered with a cover glass. After focusing with the micro-

scope upon a certain cyst on the slide, then pressing hard upon the cover with forceps, pressure was exerted on the cysts. In some instances the cyst ruptured with the extension of protoplasmic material but typical "buds" were not produced. Portions of the washed cysts were heated to boiling in different solutions, then examined under the microscope. No "budding" was observed after boiling in water, ether, 95% alcohol, glacial acetic acid, Schaudinn's fluid, or Allen's B_3 fixative, but "buds" were found after boiling in the picromercuric fixative. Upon careful examination of the fixed and stained slides it was found that the nuclear structure in the cysts more frequently resembled that of *E. coli* than it did *C. lafleuri* but that "buds" and chromophile ridges occurred in both kinds, especially in the slides fixed with picromercuric. This indicates that "budding" and chromophile ridges may also be induced in the cysts of *E. coli* by the action of the fixatives (Fig. 7). In some cysts on these slides the nuclear structure was rather intermediate between that typical for *E. coli* and that typical for *C. lafleuri*. Such a cyst is illustrated in figure 6.

In another series of slides (Case no. 57) from a stool containing *E. coli*-like cysts, the action of Schaudinn's fluid at different temperatures was also tried out. Some slides were fixed at room temperature and some hot (about 60° C) and others in B_3 fixative as a control. Upon examining these slides it was found that most of the cysts showed no modifications. Some that had been fixed in hot Schaudinn's fluid were distorted but in none was there any evidence of "budding."

The next case was a host in New Mexico (Case no. 85) and the specimen received contained great numbers of eight-nucleated cysts. No vegetative forms were present. A series of slides from this stool was made, again using Schaudinn's fluid and picromercuric. Upon examination it was found that regardless of the fixative all of the slides showed "budding" cysts, but the cysts on the slides fixed in Schaudinn's showed "budding" or a chromophile ridge in only 10 per cent of the cysts counted, while 90 per cent of the cysts on the slides fixed in picromercuric gave evidence of "buds" or ridges. These percentages are based upon a count of one hundred cysts on each slide. The cysts were not all spherical in contour but were quite variable assuming spherical, elongated and ellipsoidal shapes. A chromophile ridge (Fig. 8) such as Kofoid and Swezy describe for *Councilmania lafleuri* was present in some cysts which showed no "budding" as well as in those which showed "buds." In these cysts the nuclear membrane was thin and the pericaryosomal area stained lightly making the nuclei stand out in the darker cytoplasm very distinctly. The karyosome was generally central in position, large and dispersed. The cysts ranged from 23 to 28μ, averaging 24μ in diameter.

Because of the great numbers of cysts in this specimen and the interesting results obtained from the first series of slides a supply of

washed cysts was prepared for further experiments. The effect of heat upon these cysts was tested in the same manner as described previously (Case no. 80) but with no positive results. One cyst, but only one, with what was unmistakably a "bud" was found after heating a number from the culture in a solution of 95% alcohol and then applying Heidenhain's hemotoxylin; the cyst and the "bud" were equally stained. Picromercuric was applied to a number of other cysts, cysts which had been killed by boiling in water, here in a few cases distinct "buds" were seen. The washed cysts boiled in picromercuric showed "buds" in a much greater number.

Although the staining reactions of Congo red (1% in normal salt) on cysts of *E. coli* and *C. lafleuri* described by Berkovitz (1923) is not a satisfactory method of differentiation, it was decided to try the permeability of these cysts to this stain. The tendency of the cyst wall to take the stain was marked, all of the cysts being stained. In order to secure, if possible, vegetative amoebae the washed cysts were mixed with milk and fed to four rats which had been starved for 24 hours or more. Rat No. 1 was killed two hours after feeding and cysts were found in the small intestine 70 cm. from the stomach and 5 cm. from the cecum and also in the cecum. These cysts appeared to be still viable and in good condition, but there was no evidence of budding or other form of excystation.

Rats numbered 2, 3 and 4 were killed six, seven and twelve days respectively after feeding. In the cecum of rat No. 2 quite a few amoebae were found, along with Trichomonas and Chilomastix, and in the colon there were a few amoebae and some cysts with 2 to 8 nuclei. Fixed and stained slides were made. In the fresh condition, the amoebae were very active, thrusting out clear pseudopodia with ectoplasm sharply differentiated from the endoplasm. In amoebae watched for an hour, the pseudopodia did not become granular. In size, the amoebae ranged from 19 to 29μ averaging 23.7μ. In the stained slides the structure of the vegetative stage resembled closely that of the amoebae in the human case No. 52. Among encysted forms only one eight-nucleated cyst showed "budding" in a slide fixed with picromercuric, but there were not many cysts. The cysts exhibited variability in contour, but the nuclear structure of the eight-nucleated cysts was different from that in the cysts that had been fed. These nuclei resembled closely those of the cysts of *Councilmania decumani* described by Kessel (1924).

In the cecum of rat No. 3, killed 7 days after feeding there were fewer cysts and more vegetative amoebae, but both trophozoites and cysts resembled the stages from rat No. 2. One cyst with a "bud" was found on a slide fixed with picromercuric. Active trophozoites from this rat were mixed with finger blood in salt solution and watched

under the microscope, but ingestion of blood cells was not observed, although some erythrocytes adhered to the outside of the amoebae. Rat No. 4, killed 12 days after feeding, had large numbers of Trichomonas and Blastocystis in the cecum, but very few vegetative amoebae and no cysts. The vegetative amoebae resembled those from rats 2 and 3.

Control rats, numbered 5 and 6 from the same colony and about the same age were killed at the same time that rats 2 and 3 were killed and proved to be entirely negative for amoebae. Three more controls, Nos. 7, 8 and 9, were killed and examined. Rats 7 and 8 were negative for amoebae but rat 9 had some amoebae along with Trichomonas. These amoebae were similar in structure to those found in rats 2, 3 and 4, but were a little more sluggish and were smaller, ranging from 17 to 22μ with an average of 18μ. An examination of stained slides showed vegetative amoebae like those from rats 2, 3 and 4 and the one eight-nucleated cyst that was found had nuclei like those of the other rats.

In one set of slides of *Councilmania lafleuri* sent by Professor Kofoid no "buds" or "ridges" could be found, but in a second set there were many examples of "buds" and "ridges," although the slide had been fixed in hot Schaudinn's fluid. One cyst had a bud with three nuclei (Fig. 11). There were also "buds" on some of the Chilomastix cysts which were present on the same slide (Figs. 12 and 13).

DISCUSSION

In the previous section were recorded the results of a study of fresh material and prepared slides of human feces containing amoebae and cysts resembling *C. lafleuri* and also for comparison *E. coli* and *E. histolytica.*

Kofoid and Swezy (1921) established *C. lafleuri* on the following characters: (1) clear ectoplasmic pseudopodia sharply separated from the endoplasm and thrust out suddenly; (2) red-blood cells readily ingested; (3) peripheral chromatin in a thin layer, caryosome large, eccentric with a halo or with chromatin dispersed. In the cysts, (4) cyst-wall thicker than in *E. coli;* (5) variable profile—less often spherical that in *E. coli;* (6) less readily stained; (7) glycogen body more resistant to stain; (8) nuclei with little peripheral chromatin and large dispersed caryosome; (9) chromidial bodies less acicular than in *E. coli* in early stages—fasciculate, massed centrally in later stages and contributing to chromophile buds; (10) chromophile ridge forms bud through a pore in the cyst which detaches. Most of these distinctions have been checked and in this discussion each characteristic will be considered in order.

The amoebae in the free stage were observed most closely in the free active forms in the fresh stool of case no. 52 and correspond to

Kofoid and Swezy's description of Councilmania in regard to point No. 1.

Kessel (1923b) states that he was able to infect amoeba-free rats with *Councilmania lafleuri* and that the amoebae recovered from the rodent host have, in every case, presented no apparent morphological or racial change during the period of the experiment. Kessel (1924) also describes two other species of Councilmania, *C. muris* and *C. decumani* which are budding intestinal amoebae parasitic in rodents but in fixed and stained preparations he claims that they can be distinguished from the human type.

Although the vegetative amoebae, from the rats fed cysts from a human host (Case no. 85) resembled closely the amoebae in case no. 52, nevertheless the eight nucleated cysts found in these rats did not reveal the same nuclear structure as those of the original material. Instead of the large nuclei with little peripheral chromatin and the dispersed karyosome, the karyosome when present was a discrete round body, and the remainder of the chromatin was attached to the nuclear membrane much as is illustrated by Kessel for his *C. decumani*. It is therefore impossible to say that a successful transfer of *C. lafleuri* to the rats was accomplished. If the amoebae found in these rats were the same as those fed, then they had responded to the change in hosts by exhibiting a difference in nuclear structure in the eight-nucleated cysts.

Ingestion of blood cells was not observed here; however, this experiment was rather hastily done and would merit further investigation. Dobell (1921) says of *E. coli* that the "food vacuoles never contain red blood corpuscles." While Wenyon (1922) says, "If amoebae containing red blood corpuscles are present in the stool—they are *Edamoeba histolytica*." Gunn (1922) and Wight (1925) deny the ability of these amoebae to ingest red blood cells. Lynch (1924) describes an amoebae resembling in many respects Kofoid's Councilmania, but whose eight-nucleate cyst did not produce "buds," as ingesting red blood cells in a test tube readily on one occasion but failing to do so on another. This shows that an amoebae containing red blood cells is not necessarily *Endamoeba histolytica* as Dobell, Wenyon and others maintain.

The nuclear structure (distinction No. 3) as found on the stained slides of case no. 52 especially, was very similar to that of Councilmania described and figured by Kofoid and Swezy. It was this peculiar nuclear structure which first drew attention to the fact that it might not be *E. coli* and inspired the experiments described above. Some examples of what Kofoid describes as premitotic conditions, that is, with no distinct karyosome, were found in some of the slides (case no. 52) but the resting nucleus was observed in a great majority of the amoebae to be typical of *C. lafleuri*. Even in the binucleated amoebae both nuclei conformed to Kofoid's description. These results are contrary to those

of Gunn (1922), Wenyon (1922) and Wight and Prince (1927) all of whom maintain that no nuclear differences could be noted between the amoebae pronounced Councilmania and those pronounced *Endamoeba coli* by Dr. Kofoid's laboratory. Kofoid (1924) states that Gunn was dealing with mixed infections of Councilmania with *E. coli* and this may have been the case. More recently, Pickard in France (1928) has confirmed Kofoid's observations.

The cystic differences in these two forms seem to afford the greatest cause for disagreement. In regard to the thickness of the cyst-wall the present observations indicate that this distinction is one of degree only and unimportant. As for the shape of the cysts (No. 5) the great irregularity of shape was very noticeable especially in case no 85. The assymetrical forms were not produced, as Gunn (1922) states, by pressure on the cover slip because they were noticed in fresh preparations upon which no cover slip had been placed. No special study was made of the difference in staining reaction (No. 6) but casual observations showed no noticeable differences in staining reactions in the different cases. The resistance of the glycogen body to iodine (No. 7) was not studied.

The nuclei of the eight-nucleated cysts resembled closely those described by Kofoid and Swezy for *Councilmania lafleuri* although a few others resembled those described as peculiar to *E. coli.* If the cyst was not too heavily stained the nuclei of the cysts in most instances from case no. 85 stood out clearly as lighter areas in the cytoplasm and the karyosome appeared as a rule, large, central and dispersed (figs. 8 and 9). Gunn maintains that "there was no difference in the appearance of the karyosome in the Councilmania and *E. coli* cases" but in the observations here the two types of karyosome appear to be distinct.

Not much attention in these experiments was paid to the structure of the chromatoidal bodies (No. 9) and their appearance since these bodies are generally absent in eight-nucleated cysts, but much attention was directed to the chromophile ridge and "budding" described under distinction No. 10 by Kofoid and Swezy. It is upon this distinction that Kofoid and Swezy have laid the greatest stress and it is upon the validity of these characters that the ultimate fate of Councilmania as a new genus depends. So firmly convinced is Kofoid of the validity of this genus that another new species of intestinal amoebae, *Councilmania dissimilis* has been recently described and placed under this classification (Kofoid 1927). He has also transferred the species *tenuis* of Kuenen and Swellengrebel from the genus Endamoeba to Councilmania (Kofoid 1928).

In the present study on stained material the existence of the chromophile ridge, such as Kofoid describes, was confirmed in many of the cysts from case no. 85. This ridge was not due, as Gunn (1922) declares,

to the entrance of the stain into the creased wall of the ruptured cysts because it was noticed in many cysts which were not ruptured and which did not show "buds" (fig. 8). In such cases it probably represents a partial rupture with the outer membrane remaining intact. In fact, the ridge occurred in so large a percentage of these cysts that the assertion might be made that it is diagnostic. In a group of slides sent by Professor Kofoid and diagnosed by him as *C. lafleuri* none of the eight-nucleated cysts presented any "buds" nor was any ridge noted although over one thousand cysts were examined on these slides. In a second group of slides sent by Professor Kofoid and labeled *C. lafleuri* both "buds" and chromophile ridges were to be seen as well as "buds" in cysts of Chilomastix on the same slides. These slides illustrate the variability in respect to "budding." In some cases "buds" are numerous after using Schaudinn's fluid, in others no "buds" are seen with the same technique.

While "buds" similar to those described by Kofoid and Swezy (1921) were seen in several cases, especially after fixation with picromercuric, there was no satisfactory evidence that it is a normal biological process. In several attempts to produce excystation with fresh material no success was attained. Allen (1925) claims to have seen excystment taking place in *C. lafleuri* in cysts which were kept in Ringer's solution plus .01% dextrine, the amoebula which she observed was the only one in the cyst and she assumes that the seven others had excysted previously. The observations of Allen upon this amoebula recall those of Hegner (1925) for *E. coli,* but in the latter case only one amoeba emerged from the eight-nucleated cyst and left the cyst empty. According to Hegner, who has observed excystation in a number of intestinal protozoa, moisture and a favorable temperature (37° C) for a sufficient period of time (several hours) are the only essential features necessary to produce excystment. Pickard (1928) claims to have seen cysts "budding" in fresh preparations, but many more in fixed preparations due to osmotic pressure. In this study neither chromophile ridges nor "budding" in untreated preparations has been seen in either *E. coli* or *C. lafleuri.* Excystation, however, must occur, in some form, wherever and whenever the contents of the cyst escape.

Kofoid (1924) states that the "budding" can not be induced by heat and in this he may be right. He also states that it is not due to trauma and cannot be produced by pressure. Gunn (1922) contradicts these statements, but the results of the experiments made by the writer do no confirm those of Gunn. All of the evidence of the experiments discussed in this paper point to the fixing agent as the cause of the "budding" phenomenon.

In the material from New Mexico (case no. 85) "buds" were produced in both hot Schaudinn's fluid and in warm picromercuric The

picromercuric, however, produced many more "buds" in this series of slides than did the Schaudinn's fluid. The fact that these cysts also showed "budding" in hot Schaudinn's fluid while typical cysts of *E. coli* did not (case no. 57, also points to the probability of a certain difference or weakness of the cyst wall between the two species. Kofoid (1921) employs hot Schaudinn's fluid as a fixing agent. He also claims that "budding" has never been seen by him in anything but Councilmania and that there is never more than one nucleus in a bud. In a series of four slides (no. 72151) which he sent to Dr. Wenrich and which his laboratory had diagnosed as *C. lafleuri,* numerous cysts of Chilomastix showing buds were seen (figs. 12 and 13), also some cysts of *C. lafleuri* showing two and (rarely) three nuclei in the bud (fig. 11). Wight and Prince (1927) also present excellent photographs of budding in *E. histolytica* and *I. bütschlii,* and they have also found more than one nucleus in a bud. They have never seen "budding" in the fresh saline preparations but claim to have manufactured "buds" in 90% of the cysts by chemical means when none were found in fresh smears. *E. coli* and *C. lafleuri* according to these two men are the same organism, but they do speak of a "variety" of *E. coli* with some of the characteristics of *C. lafleuri* The chromophile ridge seems to be of as much importance as the "buds" as a diagnostic feature, but the two go together and the sum total of the evidence from this study seems to prove that the formation of the ridges and "buds" in cysts is not a biological process nor due to heat alone but depends largely upon the race, condition of the cysts, and the fixative used.

A clear understanding of the distinctions between *Endamoeba coli, Councilmania lafleuri,* and *Endamaeba histolytica* is a matter of importance in clinical diagnosis. In the past it has been the custom of pathologists and parasitologists to diagnose amoebae with clear pseudopodia as *Endamoeba histolytica.* It is obvious that, in the light of the present findings, this basis of diagnosis does not in itself distinguish *E. histolytica* from Kofoid's *Councilmania lafleuri.* Such a diagnosis should take into account the nuclear structure as revealed in properly fixed and stained material as well as the cysts, essentially as indicated in Kofoid and Swezy's list of differences which is given at the beginning of this discussion. It has been possible to confirm Kofoid's observations in nearly every respect except the pathogenicity and the biological nature of the process of "bud" formation. In case no. 85 there was a history of symptoms which could be ascribed to amoebiasis, which may be considered a partial confirmation of pathogenicity. In addition to the identification of *C. lafleuri* by Pickard (1928), Craig and Lynch offer observations which partially identify this form. Craig (1926) states, "I have observed all of the morphological features noted by Kofoid and Swezy as characteristic of *Councilmania lafleuri* in amoebae

that I considered as either *Endamoeba coli* or *Endamoeba histolytica,* but I may have been observing *Councilmania lafleuri* in a mixed infection with the other amoebae." Lynch (1924) states that the amoeba which ingested the red blood cells on one occasion but failed to do so on another resembled Kofoid's description of Councilmania in many respects having clear ectoplasm and eight-nucleated cysts, but being unable to find any evidence of "budding" of the cysts, and feeling that upon this phenomenon the validity of Councilmania rested, did not identify it as such.

From the observations so far made by the writer it appears that Kofoid is probably right in claiming the existence of an amoeba of the human intestine to which he gives the name *Councilmania lafleuri.* But, the process of "budding" has been found to be due primarily to the fixative employed. Since the validity of the genus Councilmania depends largely upon this process of "budding" and since it has been shown not to be a normal biological process it is suggested that the new amoeba which Kofoid and Swezy described (1921) and whose morphological observations have been confirmed here be called *Endamoeba lafleuri* in place of *Councilmania lafleuri.* This statement assumes, tentatively, the validity of the genus Endamoeba for human amoebae.

Since Wight and Prince (1927) apparently accept the idea of a "variety" of *E. coli* with the characters of *C. lafleuri,* it becomes largely a matter of opinion as to whether such a recognizably distinct form should be considered as constituting a different variety, a different species, or a different genus. For the reasons given above, a species designation seems to be the most reasonable.

SUMMARY

1. The presence in the human intestine of an amoeba corresponding in essentially all morphological details to the description of *Councilmania lafleuri* (Kofoid and Swezy, 1921) has been confirmed.

2. The pathogenic properties of this amoeba were not completely demonstrated although there was evidence that it may be pathogenic.

3. Amoeba were recovered from the ceca of rats to which cysts from human feces had been fed, but it could not be established that these amoebae were the same species as those fed.

4. The phenomenon of "budding" in the encysted forms of this amoeba as seen in prepared slides is not a biological process, but is due to the strength, temperature and type of fixing agent employed in the preparation of the material, and to the race and condition of the cysts.

5. Since the validity of the genus Councilmania depends primarily upon the biological significance of "budding" it is suggested that the correct name for this amoeba is *Endamoeba lafleuri,* assuming, for

the moment, that the name Endamoeba is available for these amoebae from human hosts.

References Cited

Hegner, R. W. 1927.—Excystation in vitro of Human Intestinal Protozoa. Science, 65 : 577-578.

Kessel, J. F. 1924.—The Distinguishing Characteristics of Parasitic Amoebae of Cultural Rats. Univ. Cal. Publ. Zool., 20 : 489-544.

Kofoid, Swezy and Kessel. 1924.—On the Distinctions between E. coli and C. lafleuri. Univ. Cal. Publ. Zool., 26 : 21-39.

Kofoid and Swezy. 1924.—Note on Dr. Gunn's Difficulties with Councilmania. Jour. Parasit., 10 : 153-157.

Kofoid, C. A. 1928.—Councilmania tenuis and C. dissimilis. Intestinal Amoebae of Man. Arch. Int. Med., 41 : 558-585.

Lynch, K. M. 1924.—Ingestion of Red Blood Cells by an Intestinal Amoebae with Eight-nucleated. Cyst. Amer. Jour. Trop. Med., 4 : 43-48.

Pickard, R. J. 1928.—Note sur l'infection a Councilmania lafleuri. Annales de Parasitologie, 6 : 155-163.

Wenyon 1922.—On three supposed new Endamoebae of Man. Trop. Dis. Bull., 19 : 19.

Wight, T., and Prince, L. H. 1927.—Artifacts in Endamoebae which have led to the naming of a new genus and species. Amer. Jour. Trop. Med., 7 : 287-309.

Explanation of Figures in Plate I

(All drawings made by aid of a camera lucida at a magnification of 3000 and reduced one-third in printing).

Fig. 1.—Binucleate cyst from case 52. Large glycogen vacuole in cytoplasm. Note small size of cyst.

Fig. 2.—Quadrinucleate cyst from same case as fig. 1. Nuclei with little peripheral chromatin, but with central, dispersed karyosome. Glycogen vacuole absent, protoplasm faintly stained.

Fig. 3.—Vegetative form of amoebae showing typical Councilmania nucleus. Large karyosome with halo. Peripheral chromatin lobed. Marked distinction between ectoplasm and endoplasm from material fixed within 3 minutes of passage of stool (case 52).

Fig. 4.—Vegetative form of amoeba from same case as fig. 3 but fixed 40 minutes after passage of stool. Nucleus of Councilmania type, but no distinction between endoplasm and ectoplasm.

Fig. 5.—Vegetative form of amoeba recovered from rat. Clear, hyaline pseudopodia. Nucleus of Councilmania type.

Fig. 6.—Eight-nucleated cyst from case 80, with "bud." Nuclear structure intermediate between that typical for *E. coli* and for *C. lafleuri.*

Fig. 7.—Cyst of *E. coli* (case 80) showing chromophile ridge.

Fig. 8.—Eight-nucleated cyst of amoeba from case 85. Chromophile ridge present but no "bud." Nuclei without peripheral chromatin, karyosomes dispersed.

Fig. 9.—Eight-nucleated cyst from same slide as fig. 8 showing both a ridge and a "bud." No nucleus in the "bud."

Fig. 10.—Eight-nucleated cyst from slide sent by Prof. Kofoid and diagnosed as *C. lafleuri.* Neither ridge nor "bud" shown.

Fig. 11.—Cyst from a slide (72151) loaned by Prof. Kofoid and diagnosed by him as *C. lafleuri.* Eight nuclei may be counted, three of which are to be seen in one "bud."

Figs. 12 and 13.—Cysts of Chilomastix showing "buds," from the same slide as fig. 11, loaned by Prof. Kofoid.

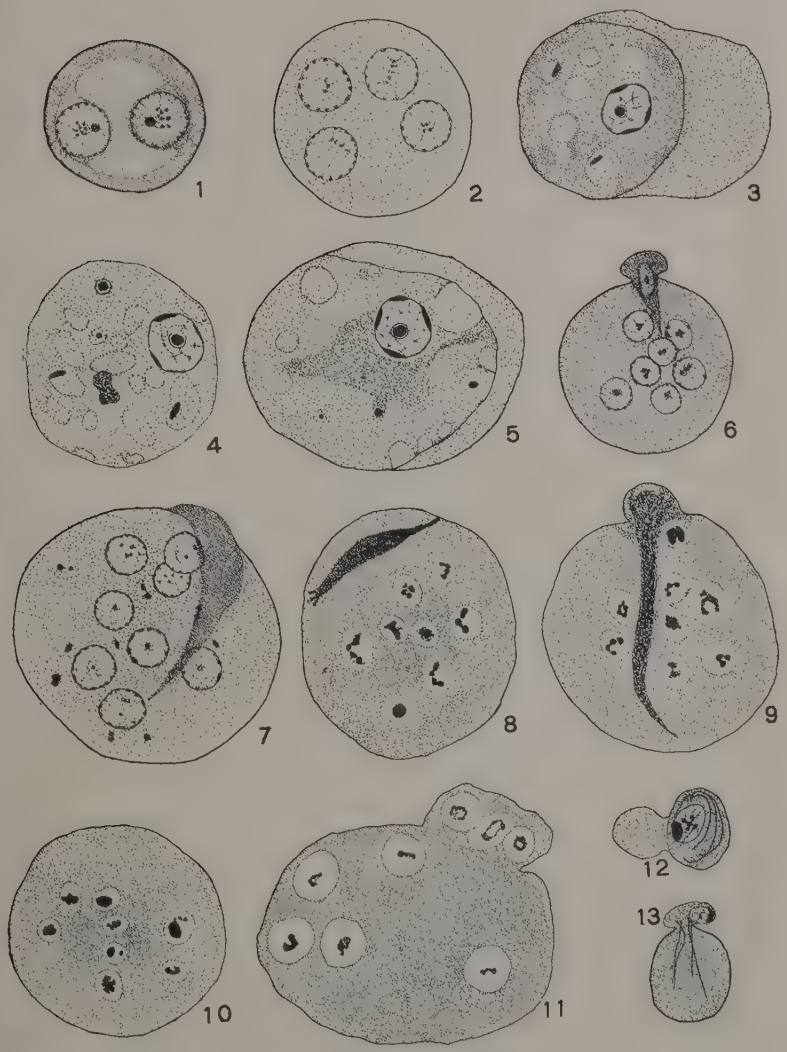

PLATE I

STUDIES ON THE DEVELOPMENT OF *ALLASSOSTOMA PARVUM* STUNKARD

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A little over a decade ago not a single life history was known for North American trematodes and up to the present time only ten, none of which concern amphistomes, have been experimentally established. An amphistome life history is here demonstrated through a study of larval and adult forms collected from the Ox-Bow, northeast of Urbana, Illinois. A cercaria from *Planorbis trivolvis* was found to encyst on crayfish and frog larvae, and when fed to bullfrogs (*Rana catesbiana*) and snapper turtles (*Chelydra serpentina*) developed into a known species, *Allassostoma parvum* Stunkard 1916. The extraordinary size of the terminal organs of the excretory system of the redia stimulated intensive studies on this system in both redia and cercaria stages.

Sincere thanks and appreciation are here extended to Professor Henry B. Ward whose guidance and criticisms have made this work possible.

THE CERCARIA

Of over 1,200 *P. trivolvis* collected during 1928 and 1929 about 8 per cent were infected with this cercaria and during June, 1928, the percentage of infection reached 19. The free swimming cercaria is large, pigmented and readily distinguishable with the unaided eye (fig. 1). With occasional intermissions at which time it attaches itself with the acetabulum and moves the anterior end of the body restlessly, it swims about continuously until it finally encysts. With the oral sucker in or near the acetabulum and the tail usually preceding the body, it makes good progress in open water.

When slightly extended the body is pyriform, pointed anteriorly. In length it ranges from 0.5 to 0.92 mm. and in width it correspondingly ranges from 0.55 to 0.26 mm. living. Under pressure of a coverglass it may reach a length of 1.6 mm. without bursting. The acetabulum opens ventrally. It is 0.19 mm. long and 0.25 mm. wide in contracted specimens and the dimentions are reversed when extended. Two large eyespots, 40μ long (deep) and 20μ in diameter at the surface, are situated just lateral to the pharyngeal pouches. Heavy pigmentation covers the anterior third to half of the body with the exception of the region of the oral sucker. Parenchymous tissue throughout the body is

* Contribution from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 350.

filled wth elliptical cystogenous granules. The tail is 1.4 to 1.7 mm. long when extended and 0.1 mm. wide at its base.

The oral sucker opens directly anteriad and in contracted specimens has an average length of 0.129 mm. and width of 0.127 mm. The anterior margin is thin and slightly ruffled. The oral sucker is provided with retro-dorsal pouches 24 to 30μ long and nearly as wide when contracted. Partially due to the heavy pigment covering them, these pouches are not conspicuous in the mature cercaria. The esophagus leaves the oral sucker as a narrow tube and widens abruptly near the bifurcation where it is surrounded by a heavy muscular pharynx 36 to 40μ long with an outer diameter of 25 to 30μ (fig. 2). Glandular cells surround the esophagus throughout its length. The intestinal crura extend laterally from the pharynx a short distance to turn abruptly posteriad and extend nearly to the acetabulum.

The beginning of the reproductive organs consists of four cell masses. The most posterior of these is slightly bilobate and lies in the median line of the body about two thirds the distance from the bifurcation of the intestine to the excretory bladder. This clump of cells is destined to become the female reproductive organs. Loosely assembled cells that stain heavily in acid stains form a line leading anteriorly to two rounded clumps, the primordia of the testes, also in the median line. The posterior testis is sometimes larger and is usually slightly to the right. The fourth clump of cells is situated between the left side of the anterior testis and the region just posterior to the pharynx. These cells are destined to become the cirrus and cirrus sac.

The excretory system is dichotomous throughout. A large pulsating bladder is situated just anterior to the acetabulum (fig. 2). It has a maximum expansion of about one third the width of the acetabulum and entirely disappears when contracted. The bladder narrows dorsally to empty through the excretory pore just anterior to the ridge separating the acetabulum from the body proper. The duct leading from the excretory bladder is joined by a caudal tube just before reaching the excretory pore. This caudal branch extends through the center of the tail about two thirds of the way towards the posterior end where it bifurcates into two elongate bladders which vary in length and proportions but are always bladder-like (fig. 1). There are no openings from these to the outside in the mature cercaria. However, in less mature larvae, still in the liver of the snail, the branches are tubiform instead of bladder-like and openings to the outside are present.

Two main trunks extend laterally from the ventral side of the excretory bladder to pass under the posterior tips of the intestinal crura. As the system of tubes is bilaterally symmetrical only one side will be described. Anteriorly from the end of the cecum the large trunk forms irregular convolutions as it progresses toward the oral

sucker. Near the pharynx the main trunk passes under the cecum and forwards, to the region of the pharyngeal pouches where it turns directly posteriad and retraces its course as far as the eye. Large crystalline spheres 4 to 10μ in diameter and highly refractive in life, fill this tube throughout its length. Lateral and posterior to the eye the main trunk bifurcates into an anterior and a posterior canal (fig. 2). The anterior canal in turn immediately divides into an anterior lateral canal which runs to the region of the oral sucker and leads to four terminal organs or flame cells; and an anterior median canal, which runs to the pharyngeal pouches and oral sucker, and leads to four terminal organs also. The posterior canal divides at once into a posterior median and posterior lateral canal. The posterior lateral canal extends along the side between the intestinal cecum and the body wall to the posterior part of the body where it turns mesad to form three or four loops, then enters the dorsal wall of the acetabulum about midway between the median and lateral lines of the body. Immediately after entering the acetabulum it divides into a short stubby branch which sends off two very small tubes which divide and lead to four terminal organs; and a longer branch which bifurcates in the most lateral region of the acetabulum into a posterior (ventral) and anterior (dorsal) branch which is joined by the corresponding tube of the other side. The posterior branch tapers abruptly and gives off two small branches which in turn divide into two each, leading to four terminal organs. After fusing, the anterior branches likewise soon taper abruptly and give off four small branches which in turn divide to extend to four terminal organs on either side, making a total of twelve on each side of the acetabulum. The posterior median canal passes under the cecum and divides in the region of the testes into an inner and outer ventral branch. The inner branch is soon lost in the pigmented area around the testes but the outer one can be traced as it runs along the ventral surface of the intestinal cecum. At about the middle of the cecum it divides into a ventral posterior canal which runs to the region between the excretory bladder and ovary, tapering abruptly to proceed as two small tubules leading to four or more terminal organs; and a dorsal posterior canal which runs laterally to the side of the body, then dorsally, lateral to and very near the posterior lateral canal. After reaching the dorsal wall the dorsal posterior canal turns back mesad and runs directly dorsal to the ventral posterior canal to the region of the excretory bladder. Here it tapers and divides into a lateral and a median canal which divide again into two smaller branches. The lateral branches run to the region posterior to the end of the cecum and branch to meet six or more terminal organs. The median branches extend toward the excretory bladder and divide to lead to four terminal organs near the bladder, and ten in the portion of the body extending

dorsally over the anterior part of the acetabulum, making a total of twenty terminal organs for the region covered by each dorsal posterior canal, and a total of forty-four or more on each side of the mature cercaria of *A. parvum* as it normally leaves its snail host. Although just forty-four terminal organs were found it seems certain that there are several more present, perhaps many. The pigmented areas into which the inner median canal and parts of both anterior canals run would be expected to have some and probably no less than twelve more. Scattered pigment and cystogenous granules in the area which the ventral posterior canal covers makes accurate counts uncertain.

THE REDIA AND MOTHER REDIA

During the warmer months the rediae are all of about the same size, having an average length of 0.8 mm., and during January, February, and March they range from very small immature individuals, about 0.3 mm. long, to large mature ones which measure 0.8 to 1.1 mm. extended. The rediae are very active when freed from the liver of their host, moving about actively in pond or rain water for more than twenty-six hours. The large ones extend from a maximum state of contraction of 0.56 mm. to a length of 1.65 mm. Pronounced annulations appear in the anterior half of the body when contracted (fig. 5). They have two pairs of ventrolateral projections which are rather effectively used in locomotion. The anterior pair is always conspicuous but the other pair is often reduced to mere bumps in the body wall. The rediae are provided with only two sets of muscles, a heavy outer circular layer and a thin longitudinal inner layer.

At the anterior end of the redia a muscular pharynx opens directly forward. In specimens with an average length of 1.1 mm. it measures 65μ long and has an outer diameter of 49μ. The esophagus is usually short and gradually enlarges into a rhabdocoel intestine which in large radiae extends to the region of the anterior locomotor organ but often fills two thirds to three fourths of the body in immature individuals. In the younger rediae and smaller mature ones a mass of irregularly arranged cells fills the area between the intestine and the pharynx. The cells in the posterior part of this mass stain heavily in hematoxylin, suggesting germ cells. Clumps of cells forming the germ balls are as common in the anterior region as they are in the posterior. Cercariae and germ balls are usually progressively arranged according to size, the largest being most anterior, but this is due to the shape of the redia and not the location of the germinal epithelium as is sometimes supposed. The birth pore is dorsal to the anterior part of the intestine and is visible only when cercariae are emerging through it.

The excretory system is essentially the same as Faust (1919) described for the redia of *C. convoluta*, Looss (1986: 183) for *Gastro-*

discus aegyptiacus, and Sewell (1922) for other members of this group. Three large terminal organs are always present on each side of the mature redia, and immature stages still within the mother redia also have this number. In individuals only 0.1 mm. long, three terminal organs on each side are always present. These six are divided into two separate systems, an anterior, median, and posterior terminal organ for each. All of these may be located by a conspicuous sinus or group of sinuses near them. Tubes extend from each terminal organ to a small bladder near the median terminal organ and the excretory pore is lateral to the bladder in or just posterior to the anterior locomotor organ (figs. 3, 5).

Mother rediae were found in snails collected during April and adult cercariae were emerging from the snails containing them. Mother rediae were not numerous and all of those observed were large and mature. They range from 1.5 to 2 mm. in length and the pharynx is larger in proportion to the size of the body than it is in the other rediae, and the intestine is also larger. Only one pair of locomotor organs is present and the entire body bears a distorted aspect (fig. 4). Young rediae within the mother redia are very active and readily distinguishable from cercariae. Rediae and cercariae were not observed developing in a single redia together.

THE TERMINAL ORGANS

The terminal organs of the redia are large and much of the structure can be seen in living material. The vibratile cones, i. e., the regions of action in the terminal organs, are triangular in outline when seen in side view and circular when seen from either end. They are somewhat retracted during rapid movement at which time they measure 6 to 8μ wide at the base and 14 to 18μ long. These organs have a very characteristic movement. Regular waves arise at the base and follow a serpentine course toward the apex. This action is somewhat like rapid peristalsis accompanied by the serpentine movement. When a specimen has been under pressure for several minutes and the activity of the vibratile cone is decreased, the serpentine action is eliminated and the movements are more nearly peristaltic in character than anything with which the writer is familiar.

The living vibratile cones appear to be longitudinally striated, but this appearance becomes less and less pronounced as movement becomes slower and entirely disappears as activity ceases. Then it would seem that the striated appearance is due to uneven surface only. The number of waves present at any one time in the vibratile cone is proportional to its activity (text figs. D, E). Very active cones have six to eight waves from base to tip while those that have almost ceased moving have but one wave and sometimes considerable interval passes before

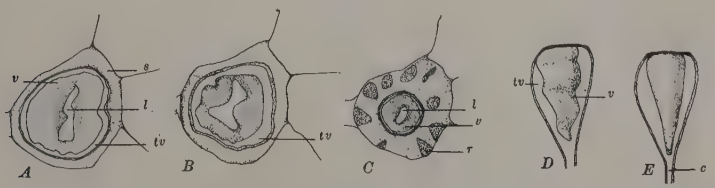
the following one appears. The apex and base are both almost stable but both may move slightly. If after action has ceased, a drop of physiological salt solution is run under the coverglass, the action is resumed in a normal manner. Optical longitudinal sections of both active and inactive cones show clear spaces running lengthwise through the center. Cones seen from either end look tubiform and are not striated even during movement. Both the movements of the active cones and the appearance of the inactive ones suggest a bulb-like structure which is more clearly shown in stained sections of fixed material.

Sections that include the long axis of the terminal organ reveal the following (fig. 6): A large spherical nucleus, 8 to 10μ in diameter, is situated near the base of the vibratile cone. Small dark granules are scattered along the periphery of this nucleus and a large nucleolus is suspended in the center. The nucleus is never in contact with the base of the vibratile cone and sometimes lies a considerable distance from it but it is always present and may thus be considered a part of the organ. The vibratile cone is clearly defined in sectioned preparations that are properly stained and is in nearly every instance extended and regular in outline. It reaches a maximum length of 21μ and basilar width of 9μ. In so far as the staining indicates, it is a homogeneous structure. Focusing up and down with the microscope reveals longitudinal light spaces in the cone similar to those seen in the living material. The vibratile cone is suspended from a heavy basilar membrane or cap into a funnel shaped vesicle which shows more distinctly in live specimens as the walls are sometimes partially or wholly collapsed against the sides of the vibratile cone in fixed material. The walls of this vesicle are continuous with the excretory tubule, thus forming a terminal vesicle. Fibrous material extends from the region of the nucleus to the excretory tubule, surrounding the basilar membrane, terminal vesicle and vibratile cone, thus forming a suspensory cup or support for the entire organ. In the walls of the suspensory cup, around the apical one-third to one-half of the organ, a number of heavy bodies show clearly. They seem to be fibrous rather than granular. Although they are slightly positive to hematoxylin, they also take picroeosin, giving them a color similar to that of the vibratile cone. Large excretory sinuses surround the organ and its accessory parts.

A study of cross sections through the terminal organs allows a number of interpretations that could not be otherwise ascertained (Text figs. A, B, C). The vibratile cone is seen here to be a true cone with a canal or lumen extending through its center from the basilar membrane to the apex. The lumen is elliptical near the base, being one-half by one-fourth the diameter of the cone and circular at the apex where it is one-third to one-fourth the diameter of the cone. The vibratile cone is free from the walls of the terminal vesicle, being attached

only at its base. Radiating bars lie in an irregular fashion in the apical region of the suspensory cup. Excepting in regions where supporting fibers attach to it the terminal organ is surrounded by excretory sinuses which have already been mentioned as conspicuous in live specimens. To summarize the arrangement of parts: a large nucleus is contained within fibrous material. This fibrous material forms a suspensory cup around the terminal vesicle which contains the vibratile cone. Radiating bars lie in the walls of the suspensory cup around the apical one-third to one-half of the vibratile cone. The terminal vesicle is continuous with the excretory tubule; and the vibratile cone is tubiform, containing a comparatively small lumen. The surrounding region is highly vesicular.

Querner (1929) has recently made a careful and detailed study of the excretory systems, giving considerable attention to the terminal organs, of *Fasciola hepatica* Looss, *Dicrocoelium lanceatum* Stiles and Hass., *Sterrhurus fusiformus* Lühe, *Paryphostomum radiatum* Dietz,



Text Figure.—*A, B* and *C*. Camera lucida drawings of cross sections through the base middle, and apex, respectively, of a terminal organ of a redia (× 1400). *D*. Diagram of a terminal organ in action (× 1100). *E*. Diagram of the same organ as shown in *D* after action has ceased. *c*, collecting tubule; *l*, lumen; *r*, radiating bars; *s*, suspensory cup; *tv*, terminal vesicle; *v*, vibratile cone.

and *Aspidogaster conchicola* Baer. This study demonstrates a remarkable similarity between the terminal organs of *P. radiatum* and those described here. The principal differences lie in the vibratile cone and the "Verstärkungsring" or reinforcement ring. The large nucleus, terminal vesicle, cap or basilar membrane and collecting capillary or tubule are very similar in the two forms. Although Querner was not able to distinguish structures characteristic of cilia, he maintains the accepted theory as to their presence. The "Verstärkungsring" is similar in position to the radiating bars but quite different in other respects. The radiating bars entirely surround the terminal vesicle but do not form a solid ring as does the reinforcement ring of *P. radiatum*.

The present investigation lends nothing in support of the conventional idea of ciliated flame cells with nucleated caps. Although it is somewhat removed from the basilar cap, a nucleus is present in the terminal organs of the redia of *A. parvum* but the presence of cilia or cilialike structures here is doubtful.

DISCUSSION

Cort (1915: 24) first called attention to the fact that amphistome cercariae are naturally divided into two sub-groups and assigned them to the subfamilies Paramphistominae Fischöder 1901 and Diplodiscinae Cohn 1904. All amphistome cercariae described since then have been placed in one of these two subfamilies; those having no retro-dorsal pharyngeal pouches, no circumesophageal sphincter, and a large cross connection between the two main trunks of the excretory system belonging to the subfamily Paramphistominae; and those conforming to the opposite condition belonging to the subfamily Diplodiscinae. Cort is right in his observation that amphistome cercariae are naturally divided into two separate sub-groups but certainly is not justified in assigning them to any of the subfamilies, for the diagnostic characteristics of the present subfamilies of the Amphistomidae are largely restricted to characters that are not present in the larval forms. Sewell (1922) also observed the natural separation of the two subgroups but proposed the names *Pigmentata* for the former and *Diplocotylea* for the latter group; and as the cercaria of *A. parvum* would by Cort's classification fall into the subfamily Diplodiscinae, whereas the adult form belongs to a different subfamily, Schizamphistominae Looss 1912, it is obvious that only Sewell's classification can be rightly used.

The cercaria of *A. parvum* is readily separated from all previously described cercaria excepting *Cercaria inhabilis* Cort 1914 and *Cercaria convoluta* Faust 1919. There is but one absolute difference between *Cercaria cortii* O'Roke 1917, but as this difference, absence of retro-dorsal pouches in *C. cortii,* is basic in classification the two must therefore be considered as separate species.

The cercaria of *A. parvum* differs considerably from *C. inhabilis* but most of the disagreements are partially or wholly explicable. *C. inhabilis* is described as moving in an unwieldly fashion (Cort 1915: 18), while the cercaria of *A. parvum* is a good swimmer; also the tail of *C. inhabilis* is 0.3 to 0.5 mm. shorter than that of mature cercaria of *A. parvum.* Under high temperatures and agitation from roughly handling the snail the cercaria of *A. parvum* sometimes emerges before fully mature and under these conditions it is unable to swim. Since Cort made his description on the basis of observations on larvae from only three snails and these were crushed, the material at his command may have been immature. The fact that he describes openings at the forked ends of the excretory canal in the tail also lends to the above conclusion for the cercaria of *A. parvum* likewise has openings there before it becomes fully mature. The excretory system of *C. inhabilis* is very incompletely described. Heavy pigmentation doubtless discouraged investigation in this direction. Even

the main trunks as described by Cort are somewhat different from those in the cercaria of *A. parvum* but the description is too incomplete to warrant a separation of the two on the basis of difference in this system. The two species differ only in respect to the features mentioned above.

Faust's description of *Cercaria convoluta* is brief and the accompanying figures are not of final value in identification. No account is given of the larva's behavior and the description of the redia is indefinite. *C. convoluta* differs from *C. inhabilis* and the cercaria of *A. parvum* in minor details only. The only delimiting characteristic given to separate *C. convoluta* from previously described species is "convolutions on the main excretory tubules" and, as has already been mentioned, it is obvious from Cort's text and figures that he made no attempt to describe accurately the excretory system of *C. inhabilis,* the only closely related species. Definite differences between *C. convoluta* and the cercaria of *A. parvum* lie entirely within the excretory system and disagreements here, in my opinion, are not numerous or important enough to allow a separation of the two. The entire system in *C. convoluta* could be superimposed upon that in the cercaria of *A. parvum* with minor adjustments. The two main trunks, the posterior lateral, and the acetabular canals would coincide throughout. The large flame cell on the posterior lateral canal in *C. convoluta* would fall in about the same position as one of the two scars that are often present in the corresponding canal in the cercaria of *A. parvum.* In the anterior region the eight terminal organs would fall upon those on the cercaria of *A. parvum* and the tubules leading to them would not differ greatly. The terminal organs in the acetabulum of the cercaria of *A. parvum* would likewise be a counterpart of twelve of the sixteen in *C. convoluta,* leaving the four most anterior to correspond to four of the neighboring group from the dorsal posterior canal, or to a group that was not found here in the cercaria of *A. parvum.* The tubules leading to the terminal organs would differ considerably, which fact can only be explained on the basis of misinterpretation on the part of either Faust or myself, which is probable in either case, for the tubules in this region are somewhat entangled and their relationships confusing (Fig. 2). Especial attention was given to the relationship of these tubules to the larger canals and in no instance were smaller units seen arising from the sides of larger ones. In the light of former descriptions by Looss (1896) and Sewell (1922) and my own observations on the form described here, it is highly probable that the excretory system of amphistome cercariae of the "Diplocotylea" group is consistently dichotomous. The median canals of the cercaria of *A. parvum* and their tributaries have no corresponding units in *C. convoluta.* The redia of *A. parvum* is similar to the redia of *C. convoluta* in every detail mentioned by

Faust. However, excretory bladders and pores were not illustrated in the figure or description of *C. convoluta* although they are doubtless present. Mother rediae were not observed by Faust.

THE ADULT

Allassostoma parvum was found in the colon and cloaca of nearly every *Rana catesbiana* and *Chelydra serpentina* collected from the given locality. It was occasionally found in *Rana pipiens* also. Several *R. catesbiana* and *C. serpentina* were periodically fed crayfish, *Cambarus (Faxonius) propinquus,* and small *R. pipiens* on which large numbers of the cercaria had encysted and the adult *A. parva* taken from these experimental hosts were easily separated into distinct groups according to their degree of maturity, and these groups were in each case easily correlated with the number of feedings and periods at which they were given.

Further studies are in progress and a more detailed account will be given in a subsequent communication.

SUMMARY

1. The cercaria, redia, and mother redia of *Allassostoma parvum* Stunkard 1916 have been found and described.

2. The terminal organs or flame cells in the redia appear to be tubiform cones rather than ciliated structures.

3. Amphistome cercaria can be separated into two natural groups but these do not conform to the present subfamilies.

4. The cercaria of *Allassostoma parvum* is probably identical with *Cercaria inhabilis* Cort 1914 and *Cercaria convoluta* Faust 1919.

References Cited

Cort, W. W. 1914.—Larval Trematodes from North American Fresh Water Snails. Jour. Parasit., 1: 65-84; 15 text figs.

1915.—Some North American Larval Trematodes. Ill. Biol. Monogr., 1: 447-532; 8 pls.

Faust, E. C. 1919.—The Excretory System in Digenea. Biol. Bull., 36: 315-321; 4 text figs.

Looss, A. 1896.—Recherches sur la Faune Parasitaire de l'Égypte. Prémière partie. Mem. Inst. Egypt. 3: 1-252; 16 pls.

O'Roke, E. C. 1917.—Larval Trematodes from Kansas Fresh Water Snails. Kan. Univ. Sci. Bull., 10: 161-180; 7 pls.

Querner, F. R. v. 1929.—Zur Histologie des Exkretionsgefässystems digenetischer Trematoden. Zeit. Parasitenkunde, 1: 489-561; 32 text figs.

Sewell, R. B. S. 1922.—Cercariae Indicae. Ind. Jour. Med. Res., 10: 1-372; 32 pls.

Stunkard, H. W. 1916.—On the Anatomy and Relations of Some North American Trematodes. Jour. Parasit., 3: 21-27.

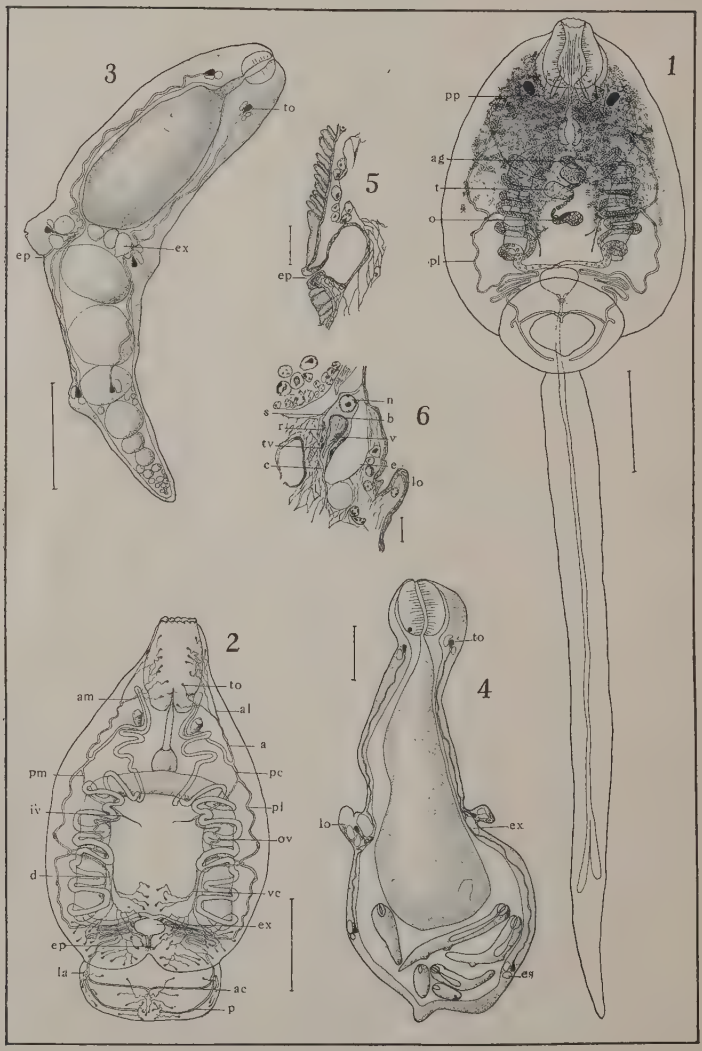

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PLATE II

Explanation of Plate II

All drawings were made with the aid of a camera lucida; in figures 1, 2, 3 and 4 the smaller units of the excretory system were inserted free hand.

The scale represents 0.2 mm. in figures 1, 2, 3 and 4; in 5 and 6 it represents 0.01 mm.

Abbreviations Used

a, anterior canal
ac, anterior acetabular canal
ag, anterior germ mass
al, anterior lateral canal
am, anterior median canal
b, basilar cap
c, collecting tubule
d, dorsal posterior canal
ep, excretory pore
es, excretory sinus
ex, excretory bladder
iv, inner ventral canal
l, lumen
lc, lateral acetabular canal
lo, locomotor organ
n, nucleus
o, ovary
ov, outer ventral canal
p, posterior acetabular canal
pc, posterior canal
pl, posterior lateral canal
pm, posterior median canal
pp, pharyngeal pouches
rb, radiating bars
s, suspensory cup
t, testis
to, terminal organ
tv, terminal vesicle
v, vibratile cone
vc, ventral canal

Fig. 1.—Ventral view of the mature cercaria.

Fig. 2.—Dorsal view of the mature cercaria under slight pressure of coverglass.

Fig. 3.—Dorsal view of the redia.

Fig. 4.—Dorsal view of the mother redia.

Fig. 5.—Section through excretory pore of a redia.

Fig. 6.—Frontal section of a redia showing a terminal organ near the posterior locomotor organ.

OBSERVATIONS ON *LEUCOCYTOZOON SMITHI;* WITH NOTES ON LEUCOCYTOZOA IN OTHER POULTRY

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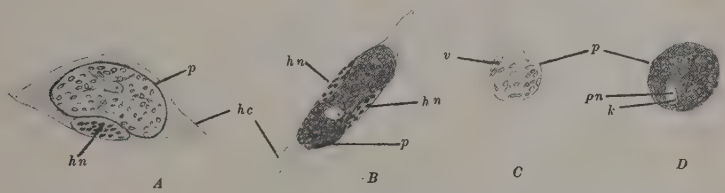
One of the most noteworthy contributions in its time to the study of the life cycles of protozoa was Schaudinn's famous memoir on the life cycles of the parasites of *Athene noctua.* Even though Schaudinn's conclusions proved erroneous, at least they served as an impetus for renewed attention to the morphology and life history of hematozoa. My recent discovery of a focus of Leucocytozoon infection in Minnesota and North Dakota should therefore prove of general interest to American protozoologists. The counties involved were Clay (Minn.), Cass (N. Dak.), and Pembina (N. Dak.), all the infected areas being in the Red River valley, the basin of a glacier lake (Lake Agassiz) in prehistoric times. The Leucocytozoon I have encountered there is *Leucocytozoon smithi,* Laveran et Lucet 1905, parasitic in the domestic turkey.

This parasite was discovered by Theobald Smith in 1895 in the Eastern United States; it has also been reported by Laveran and Lucet in 1905 from France, by Wickware in 1922 from Eastern Canada, by Stephan in 1922 from Germany, by Knuth and Magdeburg in 1924 from Germany, by Yakimoff and Rastegaieff in 1927 from the Crimean peninsula, and by Knuth and David in 1928 from Germany. The absence of a description of *L. smithi* in the American parasitological literature, the extreme scarcity of observations of Leucocytozoa in domestic birds, and the strange fact that *L. smithi,* in these 35 years following its discovery, has never been reported from the United States, prompted me to record my observations on this parasite.

In the present paper I confine myself to the sexual stage of the parasite as seen in the peripheral blood. For observations smears from the freshly drawn blood were used. The stain used was Wright's stain without previous alcoholic fixation of the blood film. This method seemed to give the most satisfactory contrast. The parasite stains blue, the host cell nucleus or its parts bright pink in contrast to the normal red blood cell whose nucleus stains blue. If the invaded cell is an erythrocyte, then this deviation from the normal staining affinity seems rather startling. It may be assumed that the staining affinity of the nucleus is altered by changes in the internal oxydation reduction potential due to the karyorhexis and karyolysis, undoubtedly brought about by the katabolism of the parasite. The nature of the host cell is still disputed, though it is the consensus of the majority of the later observers that it is an immature red blood cell and that other mononuclear blood

cells may occasionally also function as hosts. Dr. Taliaferro, who had the kindness to read the manuscript and to examine several slides, suggests that the host cell may be some greatly modified cell from the general reticulo-endothelium. This latter view seems to me to be the more plausible one, as it would explain the apparent health of the parasitized birds, since repair of any damage to the reticulo-endothelial system may rather easily and quickly take place without any manifest symptoms of disease, while in the case of blood corpuscles being invaded, it seems hardly imaginable, in the face of the destructive consequences to the host cell, that there should not be any outward clinical symptoms comparable to those seen in cases of avian paludism.

As a rule many forms of the parasite are seen, both intracorpuscular and extracorposcular, in the bloodsmears from turkey poults. The freed mature parasite exhibits all transitional changes from oval to round. In the host cell, however, the shape of the parasite is always oval, that is in the case of one host cell nucleus being present, the hemato-



Text Figure.—*hc,* hostcell; *hn,* hostcell nucleus; *k,* karyosome; *p,* leucocytozoon; *pn,* parasite nucleus; *v,* vacuole.

zoon, apparently yielding to the pressure from the host cell nucleus, appears bean shaped (Text fig. A). In a few cases the host cell nucleus is found lying over the gametocyte, occupying the center of the parasite; in these cases no indentation of the Leucocytozoon is visible When the nucleus is split in two parts, the parts of the host cell nucleus will be found positioned on either side of the parasite, which, yielding to the pressure of the nuclei parts, appears then in the shape of a dumbbell (Text fig. B). The nucleus may occasionally be split in three or more parts. Also two parasites have been found occupying one host cell. It may be mentioned here that the length of the host cell nucleus is invariably found shorter than that of the Leucocytozoon, usually about one third to two thirds the length of the latter. My measurements of the parasite, recorded in the table, have been made from stained preparations. The phenomenon that the parasite appears much broader in dry blood films than in the hanging drop preparation suggests, as was pointed out by Smith, that "the organism may be a flattish body, rolled up, so as to bring the two lateral margins near together."

Evidently, the natural or true form of the free mature parasite is a sphere (Text figs. C, D). While enclosed in the host cell, it yields to the pressure of the cell wall and the host cell nucleus. Presumably influences of tension differences and currents set up in the cytoplasm of the parasite (such currents have actually been described by Wenyon in the case of *L. neavei*) also play a rôle in determining the shape of the parasite. The Leucocytozoon becomes elongated. Thus the host cell has turned out to be a veritable "bed of Procrustes" for the parasite.

No less profound are the changes and the noxious action wrought on the invaded host cell and its nucleus (Text figs. A, B). The host cell stretches to about 4-5 times its normal length, the ends become drawn out in spindle shape, the host cell nucleus splits in two or more parts and finally disintegrates into minute granules (chromidia). After the rupture of the host cell nucleus, these granules surround the parasite like a band, 2μ or more wide, and sometimes are seen scattered all over the host cell protoplasma and even far up in the spindle shaped part.

The appearance of the cytoplasm of the macrogametocyte is honeycombed or alveolate. It is rather dense and stains deeply violet blue. In the microgametocyte it is somewhat less dense and stains pale blue. The size of the vacuoles varies from 0.1 to 3.5μ in diameter. This may indicate that they are of a contractile or pulsating type. When the larger size of vacuoles are present, the cytoplasm presents a more homogeneous appearance. In rare cases, a granular structure has been identified. In both sexes a nucleus can be observed. In the female parasite it does not stand out as clear cut as in the male one. As a result of currents and pressure differences in the cytoplasm, the nucleus may appear of varying shape, measuring about 5.8 by 3.6μ, with a coccoid body of about 1μ diameter, presumably the karyosome. The parasite nucleus stains Thiazinred both with Wright's and with Giemsa stain. There are no chromatin granules demonstrable in the nucleus as is the case in *L. neavei, L. sabrazesi* or *L. struthionis.*

According to the classification of M. and A. Leger, this parasite would belong to the spindle shaped type of the subdivision represented by *L. simondi.* This classification, based on differences of the invaded cell rather than on differences of the parasites themselves, is so artificial, that sooner or later, as knowledge of the Leucocytozoa increases, it will have to be abandoned in favor of a more natural one. It is usually taken for granted that Leucocytozoa cannot be identified outside their hosts. I have compiled the available data on the Leucocytozoa parasitic in domestic poultry. From an analysis of these figures (see table) it would seem that there are striking differences in size, as well as in some morphological characteristics, which would seem to make the identification of the host of any one of these Leucocytozoa in the intracellular sexual stage not so very difficult, provided the constancy of the measurements and the accuracy of the other details observed can be confirmed.

Investigator and Date	Host	Species of Leucocytozoon	Sex	Host Cell (μ)	Parasite (μ)	Parasite (μ) Nucleus	Chromatin Granules in Nucleus	Structure Cytoplasm
Smith (1895).....	Turkey	L. smithi	..	65 — 70	25 × 6			
Laveran and Lucet (1905).....	Turkey	L. smithi	..	43 — 44	14 × 8-25 × 5	Round or oval	.....	Vacuolated and granular
Volkmar (this paper).....	Turkey	L. smithi	♂	50 × 7-9	20 × 7	Oval	Absent	Alveolate
Volkmar (this paper).....	Turkey	L. smithi	♀	50 × 7-9	25 × 5.5	Oval	Absent	Alveolate
Volkmar (this paper).....	Turkey	L. smithi	♂	(Freed parasite)	10 × 9	Oval	Absent	Alveolate
Volkmar (this paper).....	Turkey	L. smithi	♀	(Freed parasite)	11 × 10	Oval	Absent	Alveolate
Wenyon (1908).....	Guinea fowl	L. neavei	..	50 × 5-10	20-25 × 5	Elongated	Present	Hyaline
Kerandel (1913).....	Guinea fowl	L. neavei	♂	.....	14-18 × 4.5-6.8	.....	Present	Granular
Kerandel (1913).....	Guinea fowl	L. neavei	♀	.....	14-18 × 5-8	.....	Present	Granular
Franchini (1924).....	Guinea fowl	L. neavei	♂	26-33 × 10-12	9-14 × 6			
Franchini (1924).....	Guinea fowl	L. neavei	♀	30-40 × 8-10	18-21 × 8-10			
Knuth and Magdeburg (1924).	Goose	L. anseris	..	13-15	5-7 × 2.5-5	♂ oval, ♀ round	Present	Volutin granules
Wickware (1915).....	Duck	L. anatis	..	35-60 × 10				
Walker (1912).....	Ostrich	L. struthionis	♂	.....	4-9 × 5-7	.....	Present	
Walker (1912).....	Ostrich	L. struthionis	♀	.....	11-15 × 9-13	.....	.....	Vacuolated and metachromin granules
Mathis and Leger (1909).....	Domest. fowl	L. caulleryi	♂	20 × 20	15.5 × 15.5	4 × 3-7 × 2	.....	Vacuolated and granular
Mathis and Leger (1909).....	Domest. fowl	L. caulleryi	♀	20 × 20	15.5 × 15.5			
Mathis and Leger (1910).....	Domest. fowl	L. sabrazesi	♂	67 × 4.34	23.87 × 3.2	.....	Present	
Mathis and Leger (1910).....	Domest. fowl	L. sabrazesi	♀	67 × 6.34	23.87 × 4.4	.....	Present	
Prowazek.....	Domest. fowl	L. schüffneri	..	Probably identical with L. sabrazesi				

All measurements in micra. For convenience of comparison, I give here my measurements of the erythrocytes in poultry: fowl 12.5 × 7.25; turkey 12.5 × 6.5; duck 12.5 × 8; goose 13.5 × 8.

Perhaps the outstanding criterion for a major division seems to be the difference in structural stress. One class, the elongated form of the parasite in the so-called spindle-shape type of host cell, owes its shape in the host cell to the elasticity of its body, yielding to the tension of the cell wall and the pressure of the host cell nucleus. The many distorted parasites, reported in smears of Leucocytozoa of the spindle-shape type, may easily be explained by the collapse and rupture of the frail parasite structure and the escape of the enchylema. The so-called round type of Leucocytozoon probably owes its appearance to the tougher consistency and greater resistance of its cytoplasma, perhaps the beginning differentiation into an ectoplasma, enabling the parasite not only to retain its shape but to exert pressure on the host cell nucleus, deforming it to the extent of creating the impression that the Leucocytozoon "has eaten into the host cell nucleus."

In view of the fact that the infection fades out toward winter and that the incidence and prevalence of infection varies with different years, it seems highly improbable that the intermediate host would be a parasite living in commensalism with the turkey. On the other hand, on inquiry into the topography of the land where the infected birds are raised, one will find that they come from farms with poor drainage; this, together with the above mentioned seasonal dependence of the parasite, will lead to the conclusion that the alternating host for the continuance of the life cycle of *L. smithi* must be searched among the insect fauna peculiar to these swampy regions.

Reference Cited

Wenyon, C. M. 1926.—Protozoology. 2 vols., 1563 pp., 565 figs., 20 colored pls. New York, William Wood & Co.

Exact references to authors named can be found in the second volume of Wenyon's book under Leucocytozoon, p. 903-909, and in References to Literature p. 1417-1505.

OBSERVATIONS ON THE LIFE HISTORY OF A MARINE LOPHOCERCOUS CERCARIA*

OLIVER R. MCCOY

In the course of a study of marine trematode life histories at Tortugas, Florida, a binoculate lophocercous 'monostome" cercaria commonly occurring in *Cerithium litteratum* Born, was found experimentally to continue its life cycle by encysting in the fins and underneath the scales of small fish. After encystment a number of the adult structures, which were not present in the cercaria, developed in the metacercaria. Most notable of these was a single row of 27 spines which appeared in a complete circle around the mouth. Although the adult has not yet been determined, on the basis of the structure of the metacercaria the cercaria probably is the larva of a member of the genus Acanthochasmus Looss. A preliminary report of the observations in this paper has already been published (McCoy, 1928).

The cercaria used in these life history experiments was first found at Tortugas by Miller (1926) and was described by him as Cercaria P in his preliminary report on the behavior of Tortugas cercariae. This description, although brief, contains all the details essential to the identification of the species. In the present paper, however, the cercaria will be described more completely and given the name *Cercaria floridensis* sp. nov.

Cercaria floridensis (Fig. 1) is a lophocercous cercaria belonging to the Pleurolophocerca Group of Sewell (1922: 23). It shows all the distinguishing characteristics of the group, namely, an oral sucker modified into a protrusible organ for penetration, a pair of pigmented eye spots, conspicuous penetration glands, fin-folds on the tail, and the absence of a ventral sucker and digestive tract. The cercaria is an intermittent but very rapid swimmer and reacts to a number of light and mechanical stimuli. A detailed study of its behavior has been reported by Miller (1926; 1927).

The tail of *C. floridensis* is more than twice as long as the body and is provided with a well-developed dorso-ventral fin-fold which extends along the posterior three-fourths of the tail on the dorsal side, continuing around the tip and along the posterior third on the ventral side. At its widest point, the dorsal fin is about as broad as the tail, but

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ventrally it is only about half that wide. There is also a pair of inconspicuous lateral fin-folds on the anterior third of the tail. The oral sucker, which occupies about the anterior fifth of the body, is actively protrusible and although definitely distinct from the rest of the body, does not have the typical appearance of a sucker. A small mouth is located at the anterior tip slightly on the ventral side. No pharynx or digestive tract was distinguished. A number of very small spines are present on the anterior tip of the body around the mouth, but the rest of the body and the tail are devoid of spines. Immediately posterior to the oral sucker is a pair of prominent, darkly-pigmented eye-spots. A small amount of pigment is scattered over the dorsal surface of the anterior third of the body but it is not conspicuous.

The central part of the body (Fig. 2) is occupied by a group of fourteen large, granular glands whose nuclei may be counted with difficulty under high magnification. Ducts from these glands pass anteriorly in four bundles and open on the dorsal lip of the mouth. The two median bundles, which are located dorsally, each contain four ducts, while the two lateral bundles are made up of three ducts each. Immediately posterior to the glands is a small round mass of cells which represents the rudiment of the ventral sucker. A large thick-walled excretory vesicle occupies the posterior part of the body. When expanded, it has a shortened Y-shape. The flame cell pattern was not determined. Along the sides of the body posterior to the eye spots are numerous small glandular cells probably cystogenous in character.

The cercariae develop in small, colorless, sac-like rediae ranging up to 0.9 mm. in length (Fig. 3). The rediae do not possess any collar or locomotor appendages, but a well-developed pharynx 30μ in diameter is present. The short, rhabdocoele gut, however, is degenerate and could only be distinguished in a few specimens. Miller reported the excretory system of the redia to be composed of four flame cells on each side, two emptying into an anterior and two into a posterior collecting tubule. As many as 15 cercaria were seen developing in a single redia but apparently they do not remain inside the redia to complete their development, for when the digestive gland of an infested snail was teased apart, immense numbers of cercariae, not all completely developed, were found free in the tissues.

The average measurements taken from ten cercariae killed by gentle heat were as follows: body, 192μ by 61μ; tail, 434μ by 26μ; and oral sucker, 40μ in diameter.

Specific diagnosis of *Cercaria floridensis:* binoculate lophocercous cercaria from *Cerithium litteratum.* Fin-fold along posterior three-fourths of tail dorsally and along posterior third ventrally; also narrow lateral fin-folds on anterior third of tail. Pigmented

eye-spots present but no pharynx or digestive tract visible. Oral sucker in form of protrusible anterior organ; ventral sucker rudimentary. Small spines on anterior tip of body only. Fourteen large glands in center of body; ducts in four bundles emptying on dorsal lip of mouth. Large excretory vesicle in posterior part of body. Development in simple rediae without collar or locomotor appendages.

Cercaria floridensis continues its life cycle by encysting in the fins and underneath the scales of small fish. Almost any species of fish may serve as the intermediate host, for experimentally the cercariae have been found to encyst in the following twelve species of fish representing the most common genera present on the reef where the infested snails were collected: *Eupomacentrus analis, Abudefduf saxatilis, Neomaenis apodus, Neomaenis synagris, Haemulon sciurus, Iridio bivittatus, Blenius cristatus, Malacotenus moorei, Scarus croicensis, Lagodon rhomboides, Sparisoma flavescens,* and *Ocyurus chrysurus.* No other species of fish were exposed. Heavy infestations of the fish with the cysts were obtained by exposing them for one-half hour to a large number of the cercariae in about 100 cc. of water. When such a small amount of water was used, it was necessary to bubble oxygen through it in order to keep the fish alive. After exposure the fish were transferred to an aquarium, and the development of the metacercaria was studied over a period of 38 days.

In the experimentally infested fish, encystment took place within 12 hours after the penetration of the fish by the cercaria. The cyst was thin-walled and transparent, and was considerably larger than the contained metacercaria (Fig. 4). The cyst did not grow appreciably after it was formed; cysts 40 hours old averaged 156μ by 128μ in size, while cysts which had been in the fish for 22 days measured 166μ by 133μ. The cysts were found almost exclusively in the fin rays and underneath the scales of the fish, but occasionally cysts occurred in other parts of the body—in the muscles and around the gills. The cysts which occurred underneath the scales were more nearly spherical than those in the fins, the average measurement for four specimens 9 days old being 157μ by 150μ. The cyst wall became thicker after the cyst had been in the fish for some time, but it always remained transparent and never became pigmented.

The development of the metacercaria was not very rapid. The only changes from the cercaria to be noted in the metacercaria five days after encystment were the appearance of a pharynx at the level of the eye spots and the disintegration of the 14 large glands located in the central part of the body. The small mass of cells in the posterior third of the body appeared more prominently and could be recognized as the rudiment of the ventral sucker. The whole body had an opaque appearance and was only sluggishly motile inside the cyst.

After nine days, the body of the metacercaria had become slightly lengthened (Fig. 5). A digestive tract had appeared, with the esophagus forking near the middle of the body and the ceca extending to the posterior end. The ceca contained large refractive bodies cuboidal in shape. The eye spots had begun to disintegrate but masses of the pigment were still plainly visible. At this stage the ventral sucker definitely had the typical appearance of a sucker, but it was very small, measuring only 27μ in diameter. The oral sucker was much larger, 45μ by 50μ. The shape of the excretory vesicle was strikingly different from the form present in the cercaria; the stem was long and thick-walled and there were lateral arms which extended anterior to the ventral sucker. There was no notable accumulation of concretions in the excretory vesicle.

In a metacercaria dissected from the cyst 12 days after the penetration of the cercaria, a single row of spines was noticed developing in a circle around the mouth. The spines were considerably larger in a metacercaria 15 days old and measured about 8μ in length. At this stage the eye spots had completely disappeared but there were no other important changes in structure. By the end of 22 days, the oral spines were 15μ by 3μ in size and were more prominent. They were 27 in number and were arranged in a single uninterrupted circle around the mouth. When seen in lateral view, the spines were somewhat curved and appeared much broader at the base (Fig. 6). A few rows of small spines were present in between and posterior to the larger spines. The rest of the body of the metacercaria, however, was devoid of spines.

Three weeks after the penetration of the fish, the metacercaria (Fig. 7) had apparently attained its complete development, for only a slight increase in size and no changes in structure were noted in specimens two weeks older. A dim mass of very small excretory concretions had accumulated in the excretory vesicle the lateral arms of which extended to the anterior edge of the pharynx. The pharynx, which was located at about the anterior third of the body, was nearly as large as the ventral sucker, which occupied a position in the middle of the body. The oral sucker was very large in proportion to the ventral sucker and was distinctly funnel-shaped, with the open end directed anteriorly and encircled by the single row of 27 large oral spines. The metacercaria had grown considerably in length and was usually doubled up inside the cyst. The worms were quite active and frequently changed position. The average measurements from six specimens 22 days after the penetration of the fish were: body, 324μ by 74μ; oral sucker, 65μ in diameter; ventral sucker, 34μ in diameter; and pharynx, 36μ by 20μ.

One natural occurrence of a cyst identical in appearance with the cysts in experimentally infested fish was found in the caudal fin of a

small snapper, *Neomaenis synagris,* caught on the reef where *Cercaria floridensis* occurred in the snails. The structures of the metacercaria in this cyst agreed in every respect with the mature metacercariae experimentally developed from *C. floridensis.* Attempts were made to procure the adult of *C. floridensis* by feeding fish heavily infested with the cysts to several larger fish, the gray snapper, *Neomaenis griseus,* common grunt, *Haemulon plumieri,* and black grouper, *Mycteroperca bonaci,* but no adult worms were recovered.

Although the adult has not yet been determined, on the basis of the structure exhibited by the metacercaria *C. floridensis* apparently belongs in the genus Acanthochasmus as defined by Looss (1901). The characters on which this identification is based are principally the large, funnel-shaped oral sucker with the opening surrounded by a single, uninterrupted circle of spines, and the "Y"-shaped excretory vesicle with the lateral arms extending anteriorly to the region of the pharynx. The small size of the ventral sucker in proportion to that of the oral sucker and the long prepharynx and the large pharynx with the digestive ceca extending to the posterior end of the body are also characteristic of the genus Acanthochasmus. The metacercaria of *C. floridensis* also, in certain respects, resembles the genus Asocotyle of the family Heterophyidae, but the absence of the oral cecum found in Asocotyle and the presence of the long lateral arms of the excretory vesicle characteristic of Acanthochasmus make it much more probable that *C. floridensis* belongs to this latter genus rather than to the genus Asocotyle. Since the structure of the reproductive system could not be determined in the metacercaria, the identification cannot be made with absolute certainty. It is worthy of note that *Cercaria floridensis* does not show any characters which might indicate its systematic position. All of the distinguishing structures, oral spines, ventral sucker, digestive tract, and even the distinctive "Y"-shape of the excretory vesicle first appear in the metacercaria.

C. floridensis in general structure resembles a number of previously described cercariae but it probably is most closely related to *C. quadripterygia* described by Sinitsin (1911) from the Black Sea. According to the classification of Sewell (1922), both of these forms belong in the Pleurolophocerca Group of lophocercous "monostome" cercariae. There are about 12 species of cercariae which should fall into this group on the basis of the group characters defined by Sewell, but since the classification is probably an unnatural one, these forms may not necessarily be closely related. In fact, some of these cercariae apparently form a graded series with distome cercariae of the Parapleurolophocerca Group, certain of which are known to be larvae of members of the genus Monorchotrema, family Heterophyidae (Faust and Nishigori, 1926).

This study of the development of the metacercaria of *C. floridensis* throws light upon the type of life history to be expected for other similar cercaria of the Pleurolophocerca Group. A general similarity in the structure and life cycle of *C. floridensis,* the cercaria of *Clonorchis sinensis,* and cercariae of Monorchotrema may be pointed out. All show a general resemblance in bodily structure, possess eye spots and fin-folds on the tail, and continue their life cycle by encysting in the fins and underneath the scales of small fish as second intermediate hosts. A more complete knowledge of life histories will probably make possible the formation of larger natural groups among families of trematodes which at present are not known to be related.

SUMMARY

1. *Cercaria floridensis* sp. nov., a binoculate lophocercous "monostome" cercaria, is described from the marine snail, *Cerithium litteratum,* at Tortugas, Florida.

2. This cercaria was found experimentally to encyst in the fins and underneath the scales of small fish. Various structures which were not present in the cercaria developed in the metacercaria; most notable of these were a ventral sucker, digestive tract and a row of oral spines.

REFERENCES CITED

Faust, E. C. and Nishigori, M. 1926.—The life cycles of two new species of Heterophyidae, parasitic in mammals and birds. Jour. Parasit., 13: 91-128.

Looss, A. 1901.—Uber die Fascioliden genera Stephanochasmus, Acanthochasmus, und einige andere. Centralbl. f. Bakt. u. Parasit., 29: 628-634.

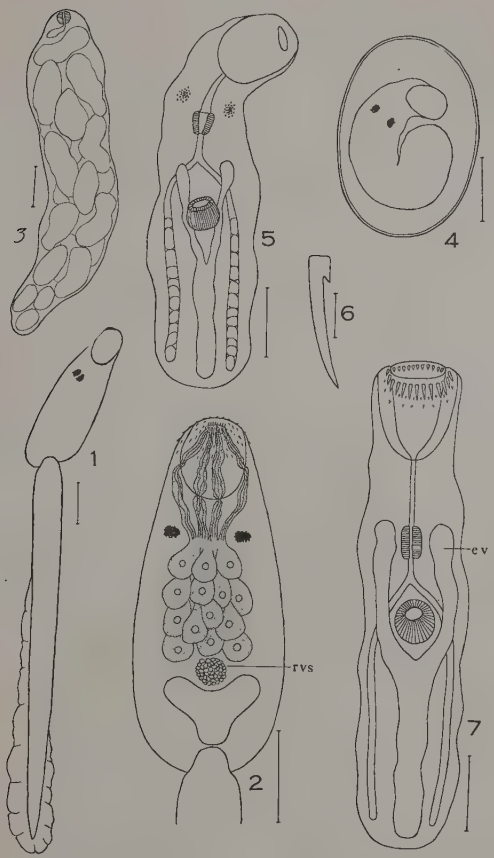
McCoy, O. R. 1928.—Studies on marine trematode life histories. Carnegie Institution Year Book No. 27: 280-284.

Miller, H. M., Jr. 1926.—Behavior studies on Tortugas larval trematodes, with notes on the morphology of two additional species. Carnegie Institution Year Book No. 25: 243-247.

1927.—Further studies on the behavior of larval trematodes from Dry Tortugos. Carnegie Institution Year Book No. 26: 224-226.

Sewell, Seymour. 1922.—Cercariae Indicae. Ind. Jour. Med. Res., 10: 1-372.

Sinitsin, D. T. 1911.—Parthenogenetic generation of trematodes and its progengy in molluscs of the Black Sea. Records of the Imperial Academy of Sciences, St. Petersburg, 8th series, 30: 1-127.



EXPLANATION OF PLATE III

All figures are diagrammatic, free-hand drawings of living specimens. The projected scale has a value of 0.05 mm. in all figures except Fig. 6; in this it has a value of 0.005 mm.

Abbreviations: ev, excretory vesicle; rvs, rudiment of ventral sucker.

Fig. 1.—Lateral view of *Cercaria floridensis.*

Fig. 2.—Dorsal view of the body of the cercaria.

Fig. 3.—Redia.

Fig. 4.—Cyst 12 hours after the penetration of the fish by the cercaria.

Fig. 5.—Metacercaria 9 days after penetration.

Fig. 6.—Oral spine in lateral view.

Fig. 7.—Mature metacercaria 22 days after penetration of the fish.

A PARAMPHISTOME FROM FISHES

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Amphistomes have been described from fishes by Daday (1907); from frogs by Cohn (1904) and Johnston (1912); from reptiles and birds by Stunkard (1917). The amphistomes of mammals have been given more consideration than those parasitic in the lower vertebrate groups. The reader is referred to the papers by Fischoeder (1903), Stiles and Goldberger (1910) and Mapleston (1923) for information on the amphistomes of mammals. The classification of this group is confused and the present status was summed up by Stunkard (1925).

The only reference, found by the writer, to amphistomes of fishes is that of Daday for South American fresh-water fishes. He described species in the genera Microrchis, Pseudocladorchis, and Chiorchis, all of which belong to the sub-family Paramphistomidae Fischoeder, 1901.

An apparently new species of amphistome, to which the name *Paramphistomum stunkardi* is given, has been found in the intestines of two species of fishes: the pumpkinseed, *Eupomotis gibbosus* (Linnaeus); and the Warmouth bass, *Chaenobryttus gulosus* (Cuvier and Valenciennes). The fishes were collected from the Eno River near Durham, N. C. This new species is placed in the genus Paramphistomum Fischoeder 1901, of the sub-family Paramphistominae. *P. stunkardi* is unique in being, so far as the author is aware, the only species of this genus known to occur in a poikilothermic animal. All amphistomes from cold-blooded vertebrate hosts have oval evaginations which are absent in the genus Paramphistomum. Paramphistomum Fischoeder 1901 and Cotylophoron Stiles and Goldberger 1910 are the two genera of amphistomes without oval evaginations. Colytophoron has a genital sucker. Since the new fluke is without oval evaginations or genital sucker, it is placed in the genus Paramphistomum, which has *P. cervi* (Schrank 1790) as its type.

PARAMPHISTOMUM STUNKARDI nov. spec.

The worms studied measured from 1.4 to 1.8 mm. in length with an average for eight worms of 1.54 mm. In breadth they were from 0.34 to 0.72 mm. with an average of 0.53 mm. The shortest worm, 1.4 mm. in length, has been taken as the type since it was the only one which contained eggs. These worms are elongate in shape and nearly cylindrical. In the living state the worms are clear, the intestinal rami being visible as yellow lines. They move slowly and were usually found in the posterior region of the host's intestine.

The large acetabulum which is subterminal in position varies in length from 0.31 to 0.54 mm.; and in breadth from 0.27 to 0.37 mm.; the average length being 0.42 mm. and the average breadth 0.35 mm. The acetabulum of the type is 0.4 mm. in length and 0.33 mm. in breadth. The opening of the acetabulum is triangular in shape with the apex directed posteriorly. The acetabular walls are covered by a well defined layer of cuticula. The cuticular covering of the worm is unarmed and forms a thick covering over the body.

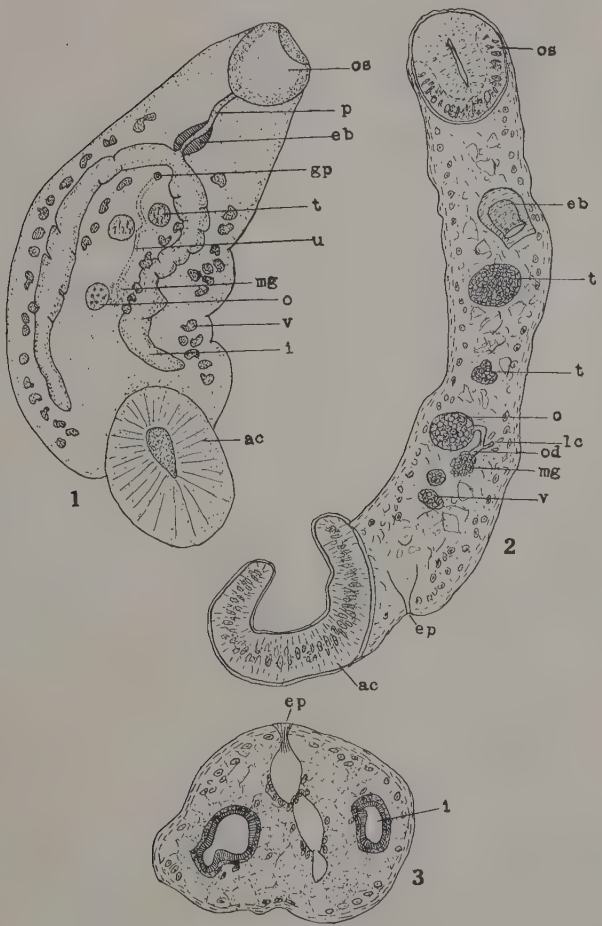
The oral sucker is terminal and ranges from ovoid to nearly spherical in shape. It is 0.16 mm. in length and 0.2 mm. in breadth, with the walls approximately 36μ in thickness. The cavity of the oral sucker is larger in its posterior region. The pharynx extends from the oral sucker and is non-muscular. Anterior to the bifurcation of the alimentary tract the walls of the esophagus enlarge to form a muscular esophageal bulb. The intestinal rami are wide, vary in diameter, and terminate in the posterior region of the body.

The ovoid, non-lobate testes are in the anterior half of the animal, being oblique in position. The anterior testes is 0.1 by 0.057 mm. and the posterior 0.114 by 0.071 mm. The vasa deferentia arise from the dorsal surfaces of the testes and extend anteriorly, uniting at the base of a large cirrus sac. The genital pore is on the ventral surface just posterior to the division of the alimentary tract.

The small ovary, 72 by 89μ is spherical in shape. The oviduct arises from the anterio-dorsal margin of the ovary and extends caudad to Mehlis' gland. Just before the oviduct passes into Mehlis' gland, Laurer's canal arises from the dorsal side of the oviduct and extends forward, opening thru the dorsal body surface anterior to the ovary. The uterus coils slightly and extends forward uniting with the male system. Few eggs are found in the uterus. In the type are four large, oval eggs, 129 by 95μ. An embryo within one of the eggs measured 95 by 66μ. The follicular vitellaria are lateral and median to the intestinal rami.

The excretory pore is in the midline of the dorsal surface just anterior to the acetabulum. A large excretory bladder lies anterior to the acetabulum and is connected with the excretory pore by a short excretory duct.

The species of Paramphistomum with the testes arranged diagonally can be divided into two groups; first, those with the opening of Laurer's canal posterior to the excretory pore; and secondly, those with this opening anterior to the excretory pore. *P. buxifrons* Leiper 1910 is the only species other than the one here described with the opening of Laurer's canal anterior to the excretory pore and testes non-lobate. *P. stunkardi* has the testes in the anterior half of the body while in *P. buxifrons* these organs are in the posterior part of the worm.



EXPLANATION OF PLATE IV

All figures were made with the aid of a camera lucida and were made from permanent mounts.

Abbreviations Used

ac, acetabulum	*lc*, Laurer's canal	*p*, pharynx
eb, esophageal bulb	*mg*, Mehlis' gland	*t*, testis
ep, excretory pore	*o*, ovary	*u*, uterus
gp, genital pore	*od*, oviduct	*v*, vitellaria
i, intestinal ramus	*os*, oral sucker	

Fig. 1.—*Paramphistome stunkardi*, ventral view × 50.

Fig. 2.—Sagittal section × 80.

Fig. 3.—Cross section × 80.

Type and cotypes have been deposited in the United States National Museum, Washington, D. C.

References Cited

Cohn, L. 1904.—Helminthologische Meittulungen II. Arch f. Naturg., 70: 112-133.

Daday, E. von. 1905.—In Sudamerikanischen Fischen lebende Trematoden-Arten. Zool. Jahrb., Syst., 24: 469-590.

Fischoeder, F. 1903.—Die Paramphistomiden der Saugethiere. Zool. Jahrb., Syst., 17: 485-660.

Johnston, S. J. 1912.—On trematode parasites of Australian frogs. Proc. Linn. Soc. N.S. Wales, 37: 285-362.

Maplestone, P. A. 1923.—A revision of the Amphistomata of mammals. Ann. Trop. Med. Parasit., 17: 115-166.

Stiles, Ch. W., and Hassall, A. 1910.—A study of the anatomy of *Watsonius* (n.g.) *watsoni* of man. Bull. Hyg. Lab., No. 60: 1-264.

Stunkard, H. W. 1917.—Studies on North American Polystomidae, Aspidogastridae, and Paramphistomidae. Ill. Biol. Mon., 3: 7-114.

1925.—The present status of the amphistome problem. Parasitology, 17: 137-148.

ON THE OCCURRENCE OF THE RAT TAPEWORM (*HYMENOLEPIS DIMINUTA*) AND THE DWARF TAPEWORM (*HYMENOLEPIS NANA*) IN MAN IN SOUTHWEST VIRGINIA*

L. A. Spindler

In connection with a study on the epidemiology of ascariasis in southwest Virginia (Cort, Otto and Spindler, 1929) certain data were gathered on the occurrence of the rat tapeworm (*Hymenolepis diminuta*) and the dwarf tapeworm (*Hymenolepis nana*) in man in this region. In a series of 2152 fecal examinations in which the Stoll dilution technic was used the eggs of *H. diminuta* were found in one case, a twelve year old girl living in an isolated rural home. This child was also infested with ascaris, the egg count being 5600 eggs per gram for each parasite. No clinical studies were made on the case and the worms were not obtained.

In contrast to the rarity of *H. diminuta* the dwarf tapeworm (*H. nana*) although not plentiful was widely scattered over the areas studied (Wise, Russell and Smyth Counties), being found in 77 (3.6 per cent.) of the 2152 examinations. The infestations with this parasite were, for the most part, confined to children (less than 14 years of age) only three cases being found in adults (those 15 years of age or over). It is interesting that about three-fourths (58) of these cases were in community groups, for the most part small mining camps, only occasional infestations being found in isolated families. It is likewise of interest that in spite of this grouping in communities there seemed to be but little tendency for the parasite to spread in families. These 77 cases were distributed among 52 different families of which there were only five with three and nine with two cases recorded. This apparent lack of spread in closely associated groups in this region is surprising especially since the parasite is generally thought to spread in families in much the same manner as the pinworm (*Enterobius vermicularis*) and has sometimes been found to be quite common in orphanages. One of the most notable examples of this condition is that described by Frey (1915) in which he found 32 per cent. of 270 children in an orphans home in Texas infested with this parasite indicating a rapid spread in such crowded conditions.

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*From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University. This study is part of the program of researches on ascariasis in children which is being conducted under the auspices of the National Research Council with the aid of a grant from the American Child Health Association.

It is a pleasure to express my thanks to Dr. W. W. Cort for his interest and advice in the work and for suggestions in the preparation of the manuscript.

In view of this situation it seemed that this parasite should be more common in families than it was found to be, especially since infestation comes about, as in the case of the pinworm, by ingestion of the eggs which are passed in the feces. In order to obtain some indication as to whether or not general soil pollution was a factor in the spread of *H. nana* in this region several experiments were run to test the length of time the eggs would remain viable on the soil. It was first necessary to determine whether it was possible to recover the eggs from soil by the isolation method used for those of ascaris and trichuris. To do this a few grams of dirt and feces containing the eggs of this parasite were mixed together and a portion treated according to the egg isolation technic of Caldwell and Caldwell which was somewhat modified in the Virginia work (Spindler, 1929). Sufficient numbers of eggs were recovered to indicate that their presence in the soil can be detected by this method. Following this a lump of feces containing hymenolepis eggs was placed on soil and set out on the roof of the laboratory where it would be exposed to the action of sun and rain. Samples were taken every day with positive results until the fourth when, although a large proportion of the feces remained, no eggs could be recovered. Similar results were obtained in all subsequent samples from this experiment. Two additional cultures were made up from fresh feces and placed on the roof and although only a small portion of each had been used by the third day it was impossible to recover eggs from either after that time. In order to carry on the same experiment under more natural conditions a fresh stool containing the eggs of *H. nana* was placed on a shaded culture plot and another on a spot exposed to the sun. No examinations were made until the fourth day when a few eggs were recovered from the shaded culture but none from the other. Examinations four days later were negative as were three subsequent examinations made on successive days.

This short series of experiments while not considered as being entirely conclusive indicates that the eggs of *H. nana* are rather non-resistant to external influences and consequently live only a short time in the soil. This opinion is strengthened by the fact that no hymenolepis eggs were recovered from a series of 13 soil samples taken from around the premises of families infested with this parasite where soil pollution was occurring. These facts indicate that general soil pollution is apparently not a very important factor in the spread of *H. nana* in this region. Consequently the parasite must depend for its dissemination either upon direct fecal contamination of food and water as in the case of the pinworm (*E. vermicularis*) or upon some agency which was not determined in this survey. In this connection Chandler (1928) observed that soil pollution was apparently not a factor in the spread of *H. nana* in India. Since the distribution of this parasite was found

to correspond very closely with that of the plague he considered the rat as being an important epidemiological factor in the spread of such infestations in that country. No evidence was obtained in this survey which would indicate that rats were responsible for the spread of this parasite in southwest Virginia.

SUMMARY

1. The rat tapeworm (*Hymenolepis diminuta*) was found in a twelve year old girl in southwest Virginia.

2. The dwarf tapeworm (*Hymenolepis nana*) was widely scattered over the region studied being found in 3.6 per cent. of 2152 examinations.

3. The eggs of *H. nana* disintegrate rapidly on the soil indicating that general soil pollution was not an important factor in the spread of this parasite in that region.

LITERATURE CITED

Chandler, Asa C. 1928.—The prevalence and epidemiology of hookworm and other helminthic infections in India. Part XII. General Summary and Conclusions. Ind. Jour. Med. Res., 15: 695-743.

Cort, W. W.; Otto, G. F., and Spindler, L. A. 1929.—Ascariasis in southwest Virginia. A preliminary report. South. Med. Jour., 22: 608-614.

Frey, J. H. 1915.—Helminthiasis at Texas State Orphans Home. Texas St. Med. Jour., 11: 229-231.

Spindler, L. A. 1929.—On the use of a method for the isolation of ascaris eggs from soil. Amer. Jour. Hyg., 10: 157-164.

A STUDY OF THE TEMPERATURE AND MOISTURE REQUIREMENTS IN THE DEVELOPMENT OF THE EGGS OF THE DOG TRICHURID (*TRICHURIS VULPIS*)*

L. A. Spindler

The eggs of the dog trichuris (*Trichuris vulpis*) which lives in the cecum and large intestine of its host are expelled with the feces and undergo a period of development on the ground. In spite of the fact that the life history of this parasite has been known for some time, little definite information is available on the factors influencing the development of its eggs. In view of this situation a study was made of the temperature and moisture requirements in the development of the eggs of this parasite.

The eggs used in this study were removed from the feces of a heavily infested dog by a method similar to that described by McCoy (1929) and kept at ice-box temperature until enough had been accumulated to carry on an experiment. Series of experiments were first run to test the temperature requirements of the eggs under favorable moisture conditions. To do this, eggs were placed in petri dishes filled with water and a few drops of 5 per cent formalin solution added to keep down bacterial growth. Cultures were then incubated at 22°, 30° and 37° C. and the rate of development at these temperatures determined. Series of experiments were likewise run to determine the minimum moisture requirements of the eggs. To do this, about equal numbers of eggs were placed on cover slips and dried in the air, enough being placed on each one to make possible a quantitative count of the various developmental stages. These experiments were carried on in much the same manner as those already reported on the eggs of the human trichurid (Spindler, 1929). When the eggs had become dry a number of the cover slips were selected at random and the eggs from half of them washed off into water which was kept at 30° C. Those from the other half were likewise placed in water which was kept at 22° C. These cultures then served as controls to test the viability of the eggs on the cover glasses which were kept at their respective temperatures. The cover glasses which were remaining were divided into equal lots and cultured under conditions of moisture and temperature to be described later.

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It is a pleasure to express my thanks to Dr. W. W. Cort for his interest and advice in the work and for suggestions in the preparation of the manuscript.

Whenever an examination was made of the eggs on cover glasses kept under any set of conditions two of the cover glasses were removed and the eggs from one washed directly into water and incubated at 30° C. as a check on their ability to develop at the time of examination. The eggs on the other cover slip were covered with water and the glass inverted on a slide. The eggs were then examined and the developmental stages of the first 100 encountered were recorded. The stages of development chosen for classification were the same as those used by Brown (1927).

DEVELOPMENT UNDER FAVORABLE MOISTURE CONDITIONS

In order to determine the rate of development of the eggs of the dog trichurid at different temperatures they were cultured in water at 37°, 30° and 22° C., and examined at regular intervals. Whenever an examination was made some of the eggs were transferred to a slide and as in the case of those on cover glasses the developmental stages of the first 100 were recorded.

In view of the fact that the development of trichuris eggs has generally been thought to be very slow the rapid development of those of the dog form at 37° is quite surprising. Five cultures were run at this temperature. In the first, due perhaps to the fact that 29 per cent of the eggs were in the early morula stage when the experiment was begun the first embryonated eggs were found on the fourth day. On the eleventh day when the experiment was terminated 84 per cent of the eggs were embryonated. In the second culture 84 per cent of the eggs were in the late morula stage on the sixth day and the first embryonated ones were seen on the eighth. On the fifteenth day, when the last examination was made, 82 per cent of the eggs were found to be embryonated. Three additional cultures were run at this temperature and the development was found to correspond very closely with that of the one just described.

Although development was found to be surprisingly rapid at 37° C., it was not materially faster than that of the eggs cultured in water at 30° C. Five cultures were run at this temperature, the last four serving as controls for the humidity experiments to be described later. In the first culture the development was more rapid than at 37° C., 93 per cent of the eggs being in the late morula stage on the third day and 13 per cent embryonated on the fifth. On the eleventh day, when the experiment was discontinued, 70 per cent of the eggs were embryonated. In the second culture 91 per cent of the eggs were in the late morula stage on the sixth day, and on the tenth 15 per cent were embryonated. On the sixteenth day, when the last examination was made, 95 per cent of the eggs were found to contain motile embryos. Development in the third culture was very similar to that in the second one,

96 per cent of the eggs being embryonated on the sixteenth day. In the case of the fourth and fifth cultures at 30 C. the development was much less rapid. In the fourth only five per cent of the eggs were embryonated on the fifteenth day. The fifth culture was continued for thirty days when only 61 per cent of the eggs were found to contain motile embryos.

In contrast to the rapid development at 37° and 30°, that of the eggs in two cultures at 22° C. was found to be quite slow. In the first, embryonated eggs did not appear until the thirtieth day and even on the fortieth only 36 per cent were embryonated. More rapid development was obtained in the second culture, however, 25 per cent of the eggs being in the late morula stage on the tenth day and 77 per cent embryonated on the thirty-fourth. In this connection it is interesting to compare the development of the dog trichurid eggs with those of the human form cultured under similar conditions (Spindler, 1929). At 30° C. about 95 per cent of the eggs of the dog trichurid became embryonated in 16 days whereas in the case of the human form only 46 per cent were embryonated in 42 days. At 22° C. the majority of the dog trichurid eggs became embryonated in 35 days while those of the human form developed more slowly, only 19 per cent containing motile embryos in 41 days. This indicates that under the conditions in which these cultures were carried on the eggs of the dog trichurid develop very much more rapidly than those of the human form.

MOISTURE REQUIREMENTS OF THE EGGS

In order to determine the minimum moisture requirements of the eggs of the dog trichurid, several series of experiments were run in which, as stated previously, eggs were placed on cover slips, dried in the air and incubated under different conditions of moisture and temperature. In the first series eggs were kept under conditions where the atmosphere was 57 per cent saturated at 22° C. and also 47 per cent saturated at 30° C.* The control eggs cultured in water at 22° and 30° readily became embryonated indicating no lack of vitality in the eggs themselves. In contrast to this those on coverslips died off quite rapidly at both temperatures. At 22°, 97 per cent were dead in eight days and at 30°, 96 per cent were dead in the same length of time, indicating that the eggs of this parasite live only a short time under such conditions. Following this, two series of eggs on cover glasses were left exposed to the air of the laboratory. In the first, 70 per cent of the eggs were dead in thirteen days and in the second 93 per cent

* The constant temperature and humidity apparatus used in these experiments had previously been devised by Dr. G. F. Otto and used by him in a large series of experiments to determine the effect of moisture on the development of various species of ascarid eggs (Otto, 1929).

were dead in fifteen days. On the other hand, the control eggs in water seemed to develop normally, indicating that the rapid death of the eggs on the cover slips was due to the conditions to which they were exposed. Since no development was obtained under these conditions, a series of eggs on cover slips was incubated in an atmosphere which was 77 per cent saturated at an average temperature of 22° C. Under these conditions the eggs died rapidly, only one per cent being alive on the sixth day and none alive on the twelfth. The control eggs cultured in water developed normally throughout the experiment.

Since the eggs of this parasite failed to develop and died under conditions where the atmosphere was partially saturated three experiments were run in which the eggs on cover slips were kept in a saturated atmosphere at both 22° C. and 30° C. The control eggs (in water) readily became embryonated in each experiment. In the first series at 30°, 95 per cent of the eggs on cover slips were dead in seven days and all were dead in twelve. Similar results were obtained in the second experiment. In the third, however, some variation was observed for which there is at present no explanation. The eggs failed to die as rapidly as in the preceding experiment, 5 per cent being alive on the sixteenth day. Of this number, 2 per cent were in the early morula and 3 per cent in the late morula stage.

In contrast to this the eggs died more slowly in the saturated atmosphere at 22° C. In the first experiment at this temperature only 7 per cent of the eggs were alive on the seventh day, but this figure remained fairly constant for several days, 6 per cent being alive in the late morula stage on the twentieth day. In the second series the death of the eggs was not so rapid, only 22 per cent being dead on the fifth day. The number of dead eggs increased from that time on until on the thirty-eighth day 76 per cent were dead and 24 per cent were embryonated. Comparable results were obtained in the last experiment, 67 per cent of the eggs being dead on the thirteenth day and 66 per cent on the twenty-first. The eggs which were alive at this time were all in the late morula stage. Since a small per cent of the eggs in the preceding experiment became embryonated, it seems reasonable to suppose that had these eggs been left a sufficient length of time some of them would have become embryonated just as in the preceding experiment. However, no evidence is yet available to indicate how long the eggs would remain alive after becoming embryonated under such conditions.

Since all of the eggs cultured on a dry medium in a saturated atmosphere at 30° C. died in a short time, it seemed possible that it might be necessary for the soil to be moist before the eggs of this parasite would complete development at this temperature. Furthermore, since only a small proportion became embryonated at 22° it seemed that similar conditions might also be necessary at this temperature, unless dry soil would

absorb enough moisture under these conditions for the eggs to become embryonated. To test this, eggs were incubated on wet and dry soil in a saturated atmosphere at 20° and 30° C. Twelve watch glasses were filled with dry soil and 4 cc. of water added to six of them.* Approximately equal numbers of dog trichuris eggs were then pipetted onto the surface of the soil in each dish. Three dishes of wet and three of dry soil were then incubated under conditions of saturation at 22° and a like number under similar conditions at 30° C. The cultures were examined by a modification of the egg isolation technic of Caldwell and Caldwell (Spindler, 1929). Control eggs were cultured in water at 30° and 22° and apparently developed normally throughout the experiment.

On wet soil at 30° C. the eggs developed quite rapidly, 68 per cent being embryonated in 29 days. Development was somewhat slower at 22°, 13 per cent of the eggs being in the early morula stage, 63 per cent in the late morula and 1 per cent embryonated in 29 days. In contrast to this, the eggs on dry soil at 30° in a saturated atmosphere not only failed to develop but also died rapidly, 98 per cent being dead in 29 days. On the other hand, although many of the eggs died on dry soil at 22°, the rate of death was not so rapid as at 30°, 29 per cent being alive in the late morula stage on the twenty-ninth day.

The results of these experiments indicate that under such conditions the eggs of the dog trichurid require a moist medium on which to complete development. In this respect it is interesting to compare these results with those previously obtained by Otto (1929) in a similar series of experiments on ascarid eggs, among which were those of the dog ascarid (*Toxocara canis*). In these experiments he found that many of the eggs of this parasite would become embryonated on slides in an atmosphere which was only 77 per cent saturated. Under similar conditions the eggs of the dog trichurid as previously shown all died in a short time, indicating that they are less resistant to desiccation than those of the dog ascarid. A somewhat similar relationship seems to exist between the eggs of the human ascarid and trichurid. For example, it has been found (Otto, 1929) that human ascaris eggs will live as long as 16 days on slides in an atmosphere 77 per cent saturated at 22° C. Contrasted to this, trichuris eggs cultured under similar conditions were observed to live only a short time, 100 per cent being dead in 12 days (Spindler, 1929). Likewise, under saturated conditions at 30° C. and 22° C. the trichurid eggs died rapidly and only a small percentage became embryonated at the lower temperature, whereas Otto found that those of ascaris would become embryonated under such conditions. This difference in the moisture requirements of the eggs has been found to have considerable practical application in accounting for the peculiar distribution of the two parasites. Since much the same moisture relationship

* This soil had previously been finely crushed and dried in the air for two days.

exists between the eggs of the dog ascarid and trichurid, it may be possible to make the same application to these parasites and account for the greater frequency of ascaris as compared to trichuris in the Baltimore dogs.

SUMMARY

1. Eggs of the dog trichurid (*Trichuris vulpis*) developed and became embryonated in from 12 to 15 days at 37° C. Development at 30° C. was nearly as rapid, the majority of the eggs being embryonated in 16 days. Development of eggs at 22° C. was much slower, 77 per cent being embryonated in 35 days.

2. When dried on cover glasses and incubated in an atmosphere 57 per cent saturated at an average temperature of 22° C., 97 per cent of the eggs died in eight days. At 47 per cent saturation at 30° C., 96 per cent died in the same time. At 77 per cent saturation at 22° C., 100 per cent died in 12 days. On slides exposed to the air of the laboratory, 93 per cent of the eggs were dead in 15 days.

3. In a saturated atmosphere at 30° C., eggs failed to develop and died in 12 days. The majority of the eggs under similar conditions at 22° died in a short time, although 24 per cent lived and became embryonated in 38 days.

4. On wet soil in a saturated atmosphere at 22° and 30°, the eggs apparently developed normally, whereas 98 per cent on dry soil at 30° died in 29 days. At 22°, 29 per cent were alive on the twenty-ninth day, withstanding conditions of dryness at 22° C. that are fatal to them at 30° C.

5. Comparison with similar experiments on the eggs of the dog ascarid (*Toxocara canis*) showed, as in the case of the human forms; that trichuris eggs require more moisture for development than those of ascaris.

LITERATURE CITED

Brown, H. W. 1927.—Studies on the rate of development and viability of the eggs of *Ascaris lumbricoides* and *Trichuris trichiura* under field conditions. Jour. Parasit., 14: 1-15.

McCoy, Oliver R. 1929.—The growth of hookworm larvae on pure cultures of bacteria. Science, 49: 74-75.

Otto, G. F. 1929.—A study of the moisture requirements of the eggs of the horse, dog, human and pig ascaris. Amer. Jour. Hyg., 10: 497-520.

Spindler, L. A. 1929.—On the use of a method for the isolation of ascaris eggs from soil. Amer. Jour. Hyg. 10: 157-164.

1929.—The relation of moisture to the distribution of human trichuris and ascaris. Amer. Jour. Hyg., 10: 476-496.

THE VIABILITY OF TRICHOMONAD FLAGELLATES IN MILK*

ROBERT HEGNER

In a recent paper the writer [1] reported a series of experiments with the human intestinal flagellate, *Trichomonas hominis,* with the object of determining its method of transmission. This organism appears to exist only in the trophozoite stage, no cyst ever having been found. It was found that most of the flagellates disappear from the fecal material kept at room temperature in from 48 to 72 hours but that a few persist for a week or more. Sufficient time is thus allowed for the transfer of viable specimens to the food or drink of man. Experiments with cockroaches and house flies demonstrated that the cockroach is probably not a transmitting agent since the flagellates are unable to pass through the digestive tract of this insect and remain alive. House flies, however, may transfer the organisms both in their vomit and feces at intervals of from twenty minutes to six hours after they are ingested. It was suggested that the contamination of food or drink by flies or unsanitary conditions accounts for the transmission of this flagellate. The dilution of fecal material containing *Trichomonas hominis* with tap water, however, demonstrated that when sufficient water is added to produce a dilution approximately as great as would result from the entrance of contaminated material into sewage or into a pond or stream brings about the destruction of the organisms within a very short time, most of the organisms being killed within an hour and none being found alive after six hours. This result appears to be due to differences in the osmotic pressure due to dilution with water. The osmotic pressure of undiluted feces was found to be 6.145 whereas that of a mixture of one gram of fecal material in 99 cc. of tap water is 0.121. No experiments were carried out at that time to determine the viability of *Trichomonas hominis* in milk. This has recently been done with the following results.

Human intestinal trichomonads of the type with five anterior flagella (Pentatrichomonas) were grown in culture and various quantities of the culture medium added to pasteurized milk. The following mixtures were set up and maintained at room temperature: (1) 10 cc. of culture to 40 cc. of milk, (2) 5 cc. of culture to 45 cc. of milk, (3) 3 cc. of culture to 47 cc. of milk, and (4) 1 cc. of culture to 49 cc. of milk. These mixtures were examined at frequent intervals during a period of

* From the Department of Protozoology of the Johns Hopkins School of Hygiene and Public Health. This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Hegner (Robert). 1928. Experimental Studies on the Viability and Transmission of *Trichomonas hominis.* American Journal of Hygiene, 8: 16-34.

eight hours and active, apparently normal, trichomonads were recovered from all of them at every examination. At the end of twenty-four hours apparently normal specimens were recovered from the mixture of 10 cc. of culture material to 40 cc. of milk and this mixture remained positive for the succeeding 24 hours. No trichomonads, however, were found in the other three dilutions at the end of 24 hours, probably because they were present in such small numbers that they could be recovered only with great difficulty. These experiments supplement the work previously reported and render practically certain the transmission of human intestinal flagellates by flies and unsanitary conditions to human beings as a result of the contamination of milk and probably through types of food and drink.

A NEW CESTODE REARED IN THE DOG*

Multiceps packii sp. nov.

Reed O. Christenson
University of Minnesota

On November 12, 1927, there was sent to me by Warden A. L. Hassell, of the Minnesota State Fish and Game Commission, a varying hare (*Lepus americanus phaenotus* Allen) taken in the vicinity of Aurora, Minnesota. A post-mortem examination showed it to be infected with a large coenurus at the tip of the left ventricle of the heart, filling the pericardial cavity, and encased in a loose connective tissue sheath. A depression at the tip of the heart suggested that the mechanical obstruction to the regular heart action was considerable. At the time the cyst was identified as belonging to the species *Multiceps serialis,* the common tapeworm cyst of jack rabbits (*Lepus campestris* Bachman) and varying hares of this region. In addition to the two species of wild rabbits given above studies were extended to the Mearns cottontail (*Sylvilagus floridanus mearnsii* Allen) which occurs in the south-eastern portion of Minnesota.

The cyst was fed to a dog which had been previously examined for helminth infection. Forty-five days after the feeding the dog died and the intestine was found packed with tapeworms. Excellent slides were prepared and studied, proving the tapeworm to be not *Multiceps serialis,* as was expected, but a species more similar to *Multiceps gaigeri* Hall, 1916. It did not, however, agree fully with the published description of this species. Through the courtesy of Dr. Hall slides of *Multiceps gaigeri* and *Multiceps serialis* were procured for comparison, and studies of these confirmed the conclusion that I was dealing with an undescribed species for which I propose the name *Multiceps packi.*

The length of the worm ranges from 36 to 60 cm., with a total of 150 to 200 segments in the strobila. The anterior portion of the chain is serrate, resembling somewhat the appearance of the serrate dog tapeworm, *Taenia pisiformis.* Posteriorly the segments elongate considerably, often tapering at both ends. About 125 segments behind the head they become quadrate and contain the developed genitalia. The mature segments include the next 20, when they pass over to those in which the developing uterine rami may be seen. The transition of these to the gravid segments includes some 30 to 40 terminal segments.

* Published with the approval of the Director as paper No. 818 of the Journal Series of the Minnesota Agricultural Experiment Station.

The scolex (Fig. 1) is that of a typical taenia, possessing four suckers and a double crown of hooks numbering from 26 to 32. The suckers are weakly protruding and measure from 200 to 250μ in diameter. In general outline the head is conical, tapering posteriorly into a relatively wide neck of 1 mm. length. The rostellum measures from 300 to 350μ, while the total diameter of the head is between 600 and 750μ. Numerous calciferous bodies occur throughout the head as well as the rest of the parasite.

The hooks of this species show no marked differences from those of some of the other species of the genus Multiceps. The large hooks have a weakly curved blade, a slightly undulatory contour to the handle, and an undivided ventral root. They measure from 140 to 150μ in length, and resemble very much those of *Multiceps multiceps,* as may be seen from the key given below. The small hooks have a similar curvature to the blade, a slightly undulatory handle, and a notched ventral root. This notch may be relatively deep or practically lacking as shown in the figure. The small hooks measure from 96 to 100μ.

The mature segments are quadrate, or longer than broad. They measure from 2.5 to 5.5 mm. in length, with a breadth of from 2.5 to 3 mm. These measurements vary, depending upon the amount of contraction of the segment. The anterior portion of each segment in the fore part of the chain is constricted, giving the serrate appearance. Some 12 to 20 segments exhibit maturity.

The male genitalia are composed of racemose testes practically filling the segment, excluding the ovarian field and a short distance anteriorly. The lobules measure from 60 to 80μ and number in the neighborhood of 300. The vas deferens arises from the anterior-median portion of the cup-like genital pore, making an obtuse angle by bending posteriorly in the region of the lateral excretory canal. It continues towards the center of the segment as a much-convoluted tube. Here it breaks up into the finer vasa efferentia. No seminal vesicle is present. The mature segment (Fig. 2) shows the vas deferens with a characteristic bend at the inner extremity of the cirrus pouch, above the prominent lateral excretory canal. Figure 4 is a camera lucida drawing of the pore region and presents the details of the cirrus, cirrus pouch, and of the reproductive tubules.

The vagina originates in the genital pore near the seminal opening. It runs mesad to the excretory canal and then bends sharply as is characteristic of the genus. It then turns posteriorly toward the seminal receptacle which is situated on the median line of the segment. The uterus extends from this point anteriorly along the middle of the segment. The vitelline gland is near the posterior margin of the segment extending laterally almost as far as the lateral extremity of the ovarian

fields. Mehlis' gland appears as a globose, weakly staining body immediately anterior to the vitelline gland. The ovaries are large; the one opposite the genital pore uniformly larger than the one nearer to it (Fig. 2). They are composed of some 20 to 30 lobules of 150μ diameter. They, too, are of racemose structure, and are united by tubules which anastomose to form the oviducts.

At the time of the death of the dog most of the segments recovered were not gravid. The indications of from 8 to 12 branches of the uterus could be clearly discerned in some of these (Fig. 3). In this segment the atrophy of the genitalia is not complete. No eggs were recovered so that details regarding them are lacking.

DEVELOPMENT

Multiceps packi develops from a coenurus which occurs in the varying hares of northern Minnesota. The cyst is not notably different from that of *Multiceps serialis*. If fed to a dog numerous worms develop, reaching the gravid condition in seven or eight weeks. It is very probable that related carnivores in the northern sections will be found to be the natural host of this parasite. Further investigations will be undertaken to determine its prevalence in the intermediate host, and its development in its primary host.

The type specimen is in the collection of the Department of Zoology, University of Minnesota. Paratypes may be found in the National Museum, and in the collection of the Zoological Division of the U. S. Department of Animal Industry.

NOTES ON THE GENUS *Multiceps*

Hall (1910) listed 7 species of the genus *Multiceps*, 2 of which had then been reared. He gave later (Hall, 1916) a description of a third species with notes on its development and anatomy. This species was reared in the dog from cysts occurring in goats in India. In a later publication (Hall, 1919) a key includes but 3 species known in the adult stage. Meggitt (1924) lists 14 possible species of the genus, most of them erected upon the basis of larval characters. Fuhrmann (1926) considers 2 species of adults in this group.

Studies of ample material of the larval tapeworm vesicles belonging to this genus have emphasized the inadequacy of available descriptions. While it is true that some of the species may be diagnosed by the morphology of the hooks alone, in others they are so similar that this is impossible. There is also such overlapping in size ranges and total numbers that these characters have little specific value. Likewise, no definite histotropisms or host specificity has been noted in the cysts in some 117 of our native wild rabbits studied. Therefore, in the following key only those forms will be included in which the life cycle is known.

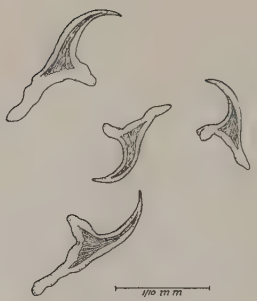

Figure A

Figure C

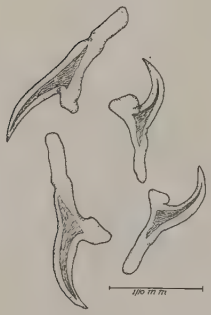

Figure CC

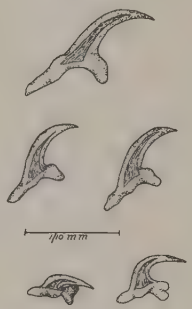

Figure D

KEY TO THE SPECIES OF MULTICEPS

(Adapted from Hall, 1919)

A. Mature segments wider than long; lateral margins scalloped by transverse furrows, posterior margins fitting glove-like over following segment. Genital papillae narrowly conical and near posterior margin of segment. Larva a coenurus with daughter bladders, found in connective tissues of rodent. *Multiceps serialis.*

AA. Mature segments longer than broad; lateral margins not marked by transverse furrows.

B. Ovaries uniformly of same size.

C. Small hooks with long curving handle, terminating in narrow distal extremity. Large hooks with tapering handle of sinuous outline. Testes do not extend posterad of ovaries to vicinity of vitellarium or between vitellarium and ovaries. Larva a coenurus in central nervous system of ungulates.

Multiceps multiceps.

CC. Small hooks with a straight handle terminating in blunt distal extremity. Large hooks with handle not tapering, and either straight and blunt, or bent dorsally at tip. Testes extend posteriad of ovaries almost to vitellarium and between vitellarium and ovaries. Larva a coenurus in central nervous system, lungs, parenchymatous organs and connective tissue of ruminants.

Multiceps gaigeri.

BB. Ovary on side opposite genital pore uniformly larger.

D. Vas deferens bending at lateral excretory canal forming obtuse angle. Testes extending posteriad as far as vitellarium. Larva a coenurus in connective tissue rodents. *Multiceps packi.*

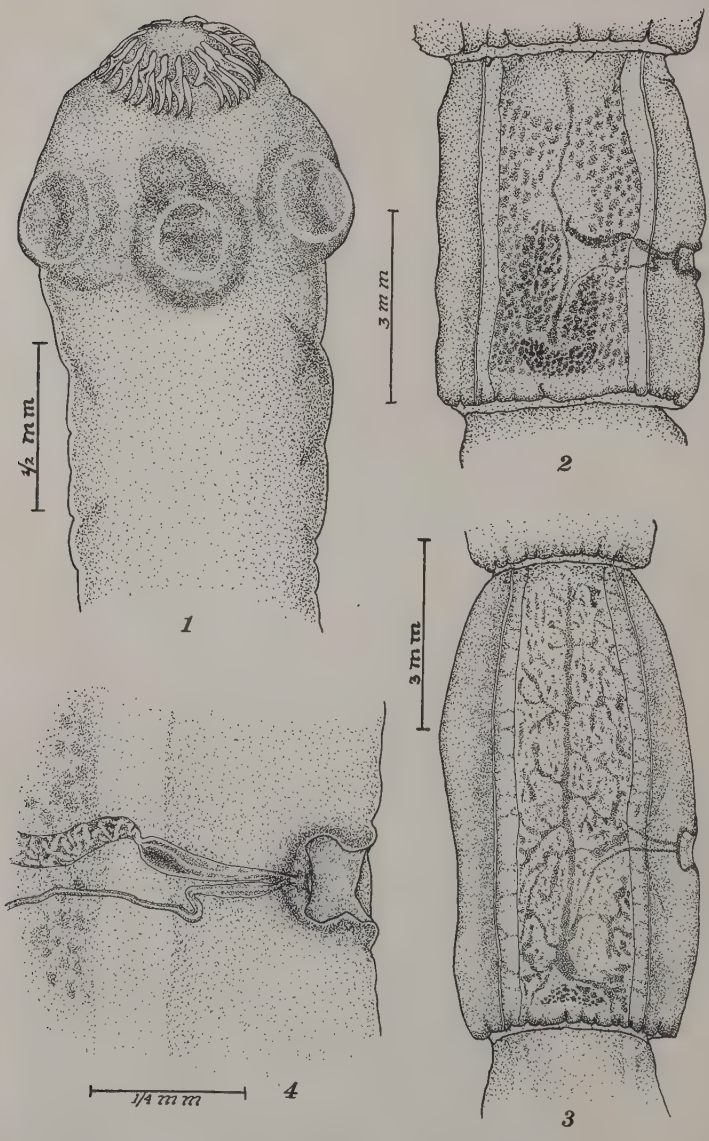

PLATE V

References Cited

Fuhrmann, O. 1926.—Catalogue des invertébrés de la Suisse. Fasc. 17, Genéve.

Meggitt, F. J. 1924.—The Cestodes of Mammals. London.

Hall, Maurice C. 1910.—The Gid Parasite and Allied Species of the Cestode Genus Multiceps. 1. Historical Review. U. S. Bur. Ani. Ind. Bull. 125, pt. 1, Wash.

1916.—A New and Economically Important Tapeworm, *Multiceeps gaigeri,* from the Dog. Jour. Amer. Vet. Med. Assoc., 50, n. s., 3: 214-223.

1919.—The Adult Taenoid Cestodes of Dogs and Cats, and of Related Carnivores in North America. Proc. U. S. Nat. Mus., No. 2258, Wash.

Explanation of Figures in Plate V.

Fig. 1.—Scolex of *Multiceps packi,* showing hooks, weakly protruding suckers, and relatively wide neck. For details of hooks see figure in key.

Fig. 2.—Mature segment: The enlarged ovary and the characteristic band of the vas deferens show clearly. The details of the cirrus, cirrus pouch, and the genital pore do not show in this figure. They are reproduced on a larger scale below.

Fig. 3.—Gravid segment: Although the uterus was not fully developed, the rami can be seen with no difficulty. These lateral rami number from 8 to 12 on each side.

Fig. 4.—Genital pore: An enlarged drawing of the pore region to show details of cirrus, cirrus pouch, and the characteristic bending of the vas deferens and vagina; the former character a specific one, and the latter characteristic of the genus.

All drawings were made with the aid of the camera lucida by Miss Grace E. Jones.

THE LENGTH OF SPECIMENS OF THE DOG HOOKWORM AFTER VARIOUS METHODS OF FIXATION *

J. Allen Scott

While measurements were being made to form a growth curve of the dog hookworm, *Ancylostoma caninum* Erc. 1859, it was found necessary to determine the effect on the length of the worms of various methods

Table 1.—*Length in millimeters of* A. caninum *fixed by different methods*

		Hot 70% Alcohol	Hot Carnoy-phenol	Water Carnoy-phenol	Hot 70% Alcohol + 20% Glycerin
Dog 416	♂	7.05 ± 0.044	.....	6.40 ± 0.099	
	♀	8.69 ± 0.092	.....	7.68 ± 0.087	
Dog 330	♂	8.36 ± 0.063	.....	8.65 ± 0.042	
	♀	10.95 ± 0.071	.....	10.96 ± 0.057	
Cat 345	♂	7.18 ± 0.061	.....	7.03 ± 0.081	
	♀	9.03 ± 0.087	.....	8.56 ± 0.055	
Dog 343	♂	5.81 ± 0.026	5.70 ± 0.038	.....	6.44 ± 0.037
	♀	8.07 ± 0.060	7.53 ± 0.055	.....	8.58 ± 0.055
Dog 345	♂	7.55 ± 0.029	7.44 ± 0.055	.....	8.00 ± 0.057
	♀	9.23 ± 0.087	8.91 ± 0.093	.....	9.05 ± 0.100
Cat 360	♂	5.57 ± 0.053	5.29 ± 0.061	.....	5.68 ± 0.095
	♀	6.25 ± 0.111	5.68 ± 0.089	.....	6.85 ± 0.094
Dog 355	♂	5.15 ± 0.075	4.97 ± 0.040	.....	5.61 ± 0.068
	♀	5.25 ± 0.103	5.19 ± 0.054	.....	6.03 ± 0.065
Dog 359	♂	7.21 ± 0.069	7.38 ± 0.064	8.19 ± 0.069	8.03 ± 0.060
	♀	9.15 ± 0.070	9.06 ± 0.013	9.95 ± 0.100	10.13 ± 0.091
		♂ ♀	♂ ♀	♂ ♀	♂ ♀
Relative.....		100 100	98 96	101 98	108 110
Percentage.....		100	97	100	109

Each average is composed of the measurements of approximately 25 worms.
Last two rows show relative lengths in percentage using hot 70 as 100%.

of fixation. The chief fixatives used for comparison were (1) hot 70% alcohol, (2) the same to which 20% glycerin had been added, (3) hot Carnoy-phenol (as described by Hetherington, 1922) and (4) cold Carnoy-phenol applied after the worms had stood over night in tap water. Immediately after fixation was complete the specimens were transferred to 70% alcohol and measured in this medium, except in the case of the glycerin-alcohol from which they were not removed. The hot fixatives were kept in the same water bath at 70°C. and the worms introduced alternately one at a time with a needle. This allowed the worms to straighten while sinking through the fixative. Fixing in

* Contribution from the Department of Helminthology, Johns Hopkins University, School of Hygiene and Public Health.

groups did not permit this straightening. All of the above methods resulted in the worms being at most only slightly curved in one plane, thus being entirely satisfactory for making an enlarged silhouette photograph for measurement with a map measure.

In Table 1 the lengths are represented by averages of approximately 25 worms of each sex selected at random from the specimens recovered from the experimental animal noted. Using the size in 70% alcohol as 100% the relative size of the other groups are given at the bottom of the table for the two sexes separately and together. It is evident that on the average the first and third groups are identical. Hot Carnoy-phenol produces slightly smaller worms on the average, but an inspection of the original figures with the probable errors will reveal that this is not significant in this series. The larger size following hot glycerin-alcohol is, however, significant.

The conclusions which arise are then that three of the methods used, namely 70% alcohol or Carnoy-phenol at 70°C. and cold Carnoy-phenol following distention in tap water, produced practically identical results; but after 20% glycerin in 70% alcohol at 70°C. the worms were nearly 10% longer. Specimens must be introduced into hot fixatives a few at a time to allow complete straightening.

Hetherington, D. C. 1922.—Some new methods in nematode technique. Jour. Parasit. 9: 102.

NOTES

THE INCIDENCE OF TRYPANOSOMES AND INTESTINAL FLAGELLATES IN AQUATIC AND TERRESTRIAL FORMS OF THE CRIMSON-SPOTTED NEWT *

ROBERT HEGNER

This is a study of the effects of a change in habitat on the transmission of two different types of protozoa. The crimson-spotted newt, *Diemyctylus viridescens,* lives for a time in the water but later takes up a residence on land. It may be infected with both intestinal and blood-inhabiting flagellates. Infection with intestinal flagellates no doubt results from the ingestion of infective stages that have been evacuated by other newts. The trypanosomes are probably transmitted by leeches that are confined to an aquatic habitat. Large numbers of both aquatic and terrestrial newts were obtained during July, 1927, at Canada Lake near Johnstown, New York. Their blood was examined for trypanosomes and their intestines for flagellates with the following results:

All of the 60 aquatic specimens examined were infected with intestinal flagellates; in 10 of them only a few were present but in the other 50 the flagellates were recorded as abundant. Of the 60 terrestrial specimens examined 58 were infected, 7 contained only a few, but in the remaining 51 intestinal flagellates were abundant.

The aquatic newts were collected from 4 stations. The blood of 2 of the 5 collected at station No. 1 was positive for *Trypanosoma diemyctyli* and that of the other 3 negative. No trypanosomes were found in the blood of 18 specimens collected at station No. 2. The 3 specimens from station No. 3 were positive and all but 2 of the 34 newts from station No. 4 were positive. No trypanosomes were found in any of the 60 terrestrial specimens examined.

These results indicate that newts retain their intestinal flagellates but lose their trypanosomes when they change from an aquatic to a terrestrial habitat. This may be explained in the following way. Under terrestrial conditions reinfection with intestinal flagellates probably takes place frequently as a result of the ingestion of food contaminated by fecal material containing infective stages, but the transmitting agent (leech?) of the trypanosomes is absent on land and hence no reinfection with these flagellates occurs. This explanation involves the assumption that the trypanosomes in the newt disappear unless the host is reinfected occasionally. Previous studies (Hegner, 1921) indicate, as a result of measurements, that the trypanosomes of the newt are all adults, and hence, unless reproduction occurs, would die off in course of time. The absence of trypanosomes in all aquatic newts from station No. 2 may also have been due to the lack of the transmitting agent since these specimens were collected from the shore of the lake where the bottom was clean and probably free from leeches, whereas the others were obtained from shallow, muddy areas where snags and weeds were abundant and leeches probably present.

REFERENCES CITED

Hegner, R. W. 1921.—Measurements of *Trypanosoma diemyctyli* from different hosts and their relation to specific identification, heredity, and environment. Journ. Parasit., 7: 105-113.

In March, 1928, was founded in Leningrad the first Russian parasitological society and thus far the only one in the Soviet. It aims to unite naturalists, students of human and comparative medicine, and those interested in applied zoology on the basis of their common interests. Professor E. N. Pawlowsky of the Military Academy of Medicine, Leningrad, was made the first president.

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BOOK REVIEWS

HYDATID DISEASE. ITS PATHOLOGY, DIAGNOSIS AND TREATMENT. By HAROLD R. DEW. 429 pp., 87 figs. The Australasian Medical Publishing Company, Ltd., Sydney.

Nothing comparable to the book under review has been put out before in this field. The well-known and valuable monograph by Dévé which is recognized as the most comprehensive treatment previously given the subject, includes in comparison with this work only a limited number of cases. The reader will recall that hydatid disease has assumed tremendous importance within the recent years since its introduction into Australia and South America and Doctor Dew has made good use of that material in his researches which have been carried on at the Hall Institute of Research in Pathology and Medicine, Melbourne. The medical literature of Australia was accessible to him as it certainly would not be elsewhere and he has studied and utilized similar material from South America. Withal he has not neglected other literature as a survey of the references seems to indicate equally complete handling of European and North American cases.

The work opens with a historical summary and this is followed by an extremely interesting discussion on geographical distribution, illustrated for Australia with valuable maps. The problems of development, general pathology, diagnostic methods, and anaphylaxis are then given successive treatment. A detailed analysis of hydatid cysts of the liver and in various other organs, together with their complications, treatment and prognosis fill two-thirds of the book. It would be impracticable here to refer to these details which are extensive and admirably compared and analyzed. Cases are illustrated by good photographs and drawings and citations to the literature are numerous.

One notices some unfortunate slips in proof-reading, like the total omission of the umlaut in German names and the consistent misspelling of the name Leuckart. These are in a way small items but they are unfortunate blemishes which acquire a conspicous character from the masterly treatment afforded otherwise. The author is to be congratulated upon having produced a work that will stand undoubtedly for a long time as the general reference text for the world in this field of work.

LEITFADEN ZUR UNTERSUCHUNG AUF DIE PARASITISCHEN PROTOZOEN DES MENSCHLICHEN DARM-KANALES. By DR. MED. F. W. BACH. 140 pp., 51 figs. Verlag von Gustav Fischer, Jena.

The author has produced in relatively brief form a complete guide to the investigation of human intestinal protozoa which will certainly be of value to workers in this field. The first part of the work deals with a description of the certain and of the doubtful species of intestinal protozoa. The descriptions are good, the comparisons are critical and the figures are particularly valuable in that they present, usually at uniform magnification and on a moderate scale, the appearances which present themselves under the microscope. Only a few are represented at higher magnifications than ordinarily employed and the delineation of observable characteristics in natural appearance makes the text a most practical aid to the individual worker.

The second section dealing with technique is extensive and will be welcomed by those who for one reason or another find it impracticable to hunt out such information from the extensive literature in this field. Much emphasis is laid upon the accurate determination of species and the precise means by which this may be achieved. The preparation of material and culture methods are both given adequate consideration as well as the ordinary microscopic investigation of routine character to which for lack of time or knowledge attention has often been confined previously.

A Manual of External Parasites. By Henry Ellsworth Ewing. 295 pp., 96 figs. Charles C. Thomas, Springfield, Ill.

Among the works recently issued by a firm which has just entered upon the field of publication in natural history is this one which deserves special attention of parasitologists. Doctor Ewing has written a most valuable and useful work on the ectoparasites. This book covers in successive sections the parasitic mites, the ticks, the biting lice, the sucking lice and the fleas. In each case is given a summary of the structure and terminology, and an outline of the taxonomy with keys to families, genera and subfamilies and with data on the control and on the literature of the particular group. As a final chapter is appended a series of descriptions of new genera of ectoparasites. This is a most unusual feature of such a work and of recognized value to those who are using a text in which classification is predominant. However, such a method of handling these descriptions is likely to be a source of difficulty to workers abroad where this book is less likely to be available than are American scientific journals. The book is attractively gotten up, well printed and reflects credit upon the publisher as well as upon the author.

Archivos do instituto biologico de defesa agricola e animal. Vol. 1, 1928. Sao Paulo, Brasil.

The attention of parasitologists should be directed to this new venture. The first number which is before us has a number of items of outstanding importance and interest for the student of medical zoology. In the initial paper, Travassos records the helminth fauna of fresh water fishes of Brazil. The descriptions are illustrated by numerous figures, mostly taken from the original sources and thus variable in style and accuracy. Of the fifty-two species studied, twenty-one are described for the first time. The protozoa of fresh water fishes in South America are described by Pinto. Special emphasis is laid upon Myxosporidia, and important data are recorded on the life histories of the various species. Other important papers: on the tineas of domestic animals, on the nematodes associated with cultivated plants and on insects as well as bacteria, make up the remainder of a volume that is impressive in appearance and reflects great credit on the institute by which it is issued.

Prof. J. Strohl of Zürich has recently written an interesting brochüre on teratology. (*Missbildungen im Tier- und Pflanzenreich.* Gustav Fischer, Jena, 1929.) His discussion has important bearings not only on such phases as the topic of parasitic castration which he discusses but also in the more general question that he considers. A student of parasitology who is interested in the causal relations in his field will find much of interest even in this brief presentation of the general topic.

The third volume from The Leiden School of Tropical Medicine (Acta Leidensia, Edita Cura et Sumptibus Scholae Medicinae Tropicae) contains among other important contributions valuable papers on studies of strongyloid and hookworm larvae and their differentiation. Also an exhaustive report on investigations in Surinam deserves special mention because of the new light thrown on the interrelations between mosquitoes and filarial diseases in the population.